

More public understanding

Everybody approves of the public understanding of science, and next week's meeting of the British Association should help to put the responsibility where it lies, on the scientific community.

THE public understanding of science enjoys the kind of esteem normally accorded to the institution of marriage, or to motherhood before that went out of fashion. The general opinion is that it is a good thing, certainly a better thing than its absence, but opinions differ on what it consists of and even on what it is for. Yet, in a modest way, the question has recently become a lively issue in Britain, where the government startled its electors and even itself by declaring, at the Williamsburg economic summit in June 1983, that it would assume responsibility for a study of the question. One tangible result is a committee of the Royal Society which hopes to report, later this year, on how the public understanding of science can be improved. Indirectly, the issue will also be rehearsed in the minds of those who attend this year's meeting of the British Association for the Advancement of Science, ten days from now.

The British Association's meeting is relevant because it is one of the vehicles by means of which, in Britain, the good cause of understanding is advanced. That this should be so is a happy accident. Begun as an alternative to the Royal Society, then largely moribund, in the heady period when the Great Reform Bill (1830 and 1832) had established the principle but not the practice of universal suffrage, and when PhD meant only what it says, the association has fortunately survived as a scientific society to which anybody may belong. Like the annual meetings of comparable organizations in North America, India (the Science Congress) and Australia and New Zealand, the British Association's jamboree is at once an occasion and an informal but not usually an exciting meeting at which the agenda is mostly about science and a means of amplifying some of the many things that are said. Its most valuable function is that it provides opportunities for scientists no longer working at the bench (perhaps because they teach in school) an opportunity to talk to people who still do, and also to bigwigs. In spite of the way in which the association's officials have come to judge the success of their annual meeting by the number of column-inches devoted to it by the popular newspapers, the question whether it adds to the public understanding of science turns on the way in which the ambiguity in that phrase is resolved.

Misunderstood

Each of the three substantive words in the phrase is used differently by different people, and none more so than "understanding". There is cause to suspect that much of the present enthusiasm for the study of the question springs from the conviction of professional scientists, especially those in managerial positions, that they and their works are at present misunderstood in the sense that well-intended activities are not publicly applauded but, instead, regarded with suspicion, even hostility. The managers of nuclear power plants and pharmaceutical companies have been in the front line for many years, and may be forgiven for yearning for a state of grace among the general public in which the plaudits they consider they deserve would indeed be heaped upon them.

Trouble has more recently descended on academic scientists (in connection with genetic manipulation) and on computer engineers (whose efficient machines nevertheless change the nature of people's jobs and sometimes get rid of them altogether). That yearning does not however deserve to be satisfied. The con-

sistent overoptimism of the management of British Nuclear Fuels Ltd (whose annual report is published this week) in the face of successive mishaps at its reprocessing plant in Cumbria, which has done as much to set back the British nuclear power programme as anything else, would have long since led to the usual penalties for incompetence if the enterprise were a private company, not a nationalized industry. So understanding does not, or should not, mean compliant acceptance.

"Science", similarly, means different things to different people. It would be wrong to think that the British Government's enthusiasm for better understanding could be required by, say, a better appreciation of the subtleties of Newton's Laws among those who now believe Newton to have been some kind of a lawyer. To the British Government just now, science is either a synonym for "wealth-creation" or a name for the curriculum content of the university courses that prepare people to be "information technologists", just as in India science means both "self-reliance" and "rural development". Everyone should beware of these flimsy definitions.

Awareness

Even what is meant by the public in the standard phrase must be looked at suspiciously. In countries such as Britain, the proportions of the population which are actually taught at school or elsewhere about science and other technical matters are substantial and growing. Given the present output of higher education, five per cent or more of the adult population will in due course be technically qualified, many of them working in technically-related jobs together with perhaps four or five times as many people who are formally unqualified. For such a population, sheer ignorance of science is not a problem — although there are important pockets of society, such as the civil service, where the effects of long-standing unnatural selection need to be countered. Awareness remains a problem, however, which on the face of things bears out the government's suspicion that the widespread acceptance of new technology could be much more enthusiastic.

The difficulty is that it is no easier to win applause for, say, information technology by conducting propaganda on a narrow front than it is to train engineers without giving those concerned an understanding of the roots of engineering in science. Even cultivated societies such as the British could, with advantage, be much more alert to the interest as well as the importance of contemporary science. Why is it otherwise?

Over the years, the British Association has made a useful contribution to this end, but the constraints on its success are telling. Although ostensibly a scientific society, and one designed for informal communication between scientists and others, the association is administratively too cumbersome to be able to tell those who attend its meetings about the current excitement in research — last year's excitement is more easily stage-managed. The association is also a perpetual prisoner of the establishment, people whose own success as scientists was achieved in the days when more general participation in the transformation of society by technology was neither feasible nor — on the face of things — desirable.

The association is still rather old-fashioned and undemocratic even by the standards of scientific societies. Part of the trouble is



the association's endless penury, which has made it depend on government hand-outs in the past decade, and has thus cramped its independence. This year the association is taking a positive step by launching a popular magazine. This move may give it a necessary and welcome measure of freedom from past convention. Let us hope so.

Understanding

More generally, and in the context of the Royal Society's inquiry into public understanding, there is also a need to break with past conventions. Avoiding the sense of being wrongly misunderstood is too narrow a platform for reform, just as castigation of the public prints for paying too little attention to science — or for paying attention and then for getting it wrong — is the wrong place from which to start. In Britain, in contrast with the United States, the chief impediment to a better public understanding of science is the reticence of the scientific community, springing perhaps from its lack of conviction that a better understanding is necessary.

There is still a tendency to divert public curiosity by *ex cathedra* declarations, to assume that the most exciting developments in science are by definition too obscure to be widely shared, to behave as if telling a wider audience about new developments constitutes an offence against one's peer group — and to be lazy. The assumption in the United States that there will be no financial support for research unless scientists take pains to tell the general public what they are about is probably false, and the practice of the precept more probably a part of the sectional competition for funds in the ring of Congress, but the British community is, by comparison with the American, tongue-tied against its own interests.

The case for changing tack is complicated. The avoidance of public discontent with innovation is neither feasible nor a legitimate part of what the scientific community should be doing. That is a task for governments and the managers of innovation, who must understand that true enlightenment about science will often make their task harder than it is by allowing a wider circle of people to know when new projects are being mismanaged. Similarly, it is not sensible to sound the case for more general understanding on the belief that democracy requires informed participation in technical decisions by the totality of voters — an informed opinion on the safety of nuclear power stations does not require first-hand knowledge of radiobiology. The simplest and most easily attainable benefit of a better public understanding would be the banishment of superstition — mistaken beliefs in things as different as astrology, the view that neutron bombs are qualitatively different from conventional nuclear weapons and assertions that this or that change in the natural environment will spell catastrophe. That would be a negative gain, so to speak. The positive side of the coin, too often neglected, is that more general participation in the sense of excitement in discovery is both a means of making sure that there will be future generations of people capable of and eager to make other discoveries and that the intellectual value of this important element of human activity will be more generally enlivening. For the rest, public understanding of science is like science itself — the course of events cannot be foretold, but their consequences are almost certain to be beneficial. □

Living without coal

Most industrial economies have become more flexible in their use of fuels since 1973.

It is nearly half a year since the coalminers of Britain came out on strike, and there is every sign that the bulk of them will continue to withhold their labour for at least as long again. Quite apart from the causes of the affair, which are at root political, the question has inevitably arisen how it can be that a fuel economy such as the British, in which coal has traditionally been the most important source of energy, could possibly have survived for as long as this without economic disaster. Is it possible that even fuel supply is

now more directly subject to pressure from market forces than it used to be?

First, of course, it is important that the coal strike began when there were substantial stocks of coal scattered around the country, especially at the electricity generating stations which are the principal consumers of this fuel. In aggregate, at the outset of the strike, there was more than enough coal in stock to keep the power stations going for half a year — since when there has been a long hot summer and a policy, inevitably expensive, of generating electricity from any fuel other than coal. It is also the case that a large part of the British coal industry, the Midlands coalfield which is a prolific source of low-quality coal, has kept working throughout the strike, causing great bitterness within the miners' union in the process. The practical result, however, is that it has been possible for the National Coal Board to maintain supplies to its customers other than the electricity generating stations, and perhaps to add modestly to its stocks at the same time. Naturally, it will be very different when the winter comes when, on past form, the average demand for electricity will be doubled, but there seems no reason why, even then, the British economy should not get by with the help of imported coal (if the striking miners and their supporters were unable to prevent this from being landed).

There are two important parts of the explanation of this state of affairs, of which the most immediate is the very great improvement during the past decade of the efficiency with which fuel is consumed. Most industrialized countries have responded in this way to the increases of petroleum prices beginning in 1973. In Western Europe, for example, the energy needed to produce each unit of Gross Domestic Product has fallen during that period by 25 per cent. In the United States, the energy-intensiveness of the economy has declined even faster. The only surprise in these developments is that they appear to have happened with so little fuss. While governments have conducted campaigns to encourage awareness of the need for economy, what has mattered in the end is that people faced with the need to pay fuel bills have elected to ensure that they will pay as little as possible.

Flexibility

At the same time, the international market in energy has become more flexible. The oil company giants in the old days, locked into special relationships with particular producing countries, behaved conservatively. Now, like many others, they find themselves buying and selling cargoes of oil on the short-term markets based on Rotterdam and the Gulf of Mexico. These informal arrangements for trading oil have flourished partly because the demand for energy, especially in the Western democracies, has been depressed during the past decade's recession, but there is no reason why they should not survive now that most economies are picking up again.

In Britain, the economy's capacity to survive without coal is further emphasized by the way in which there is also a tacit moratorium on the development of nuclear power. Although the present government announced soon after its election in 1979 that it would support a ten-year programme of nuclear construction, in reality nothing has been done. In the mistaken belief that it was bound by its predecessor's commitment to hold a public inquiry into any new construction project, the government agreed that there should be a public inquiry into the proposal to build a single pressurized-water reactor at Sizewell in Suffolk. This has now occupied more than four years already — more than two years of preparation, and eighteen months already for the formal inquiry, which cannot now be complete until the end of the year and could continue for many months yet.

During the past few months, nuclear power stations have been generating 30 per cent of the electricity supply. If there had been more of them, the proportion would inevitably have been greater. The miners profess they are on strike because of a threat that jobs will be lost as uneconomic mines are closed. What they seem not to appreciate is that they are, by their strike, providing the strongest case there could be for thinking that coal is the least satisfactory and flexible of fuels. □

US election

Heads you win, tails you win Prospects good for science

Washington

SCIENCE policy will not play a role in the presidential election campaign this year, if the two parties' platforms are any guide. Last week at the Republican National Convention in Dallas, President Ronald Reagan's party adopted a platform which devoted just four sentences to the subject, calling for a continuation of present policies and noting the 50 per cent increase in support for basic research that Reagan's Administration has brought about. The only specific proposals in the platform are for an extension of the research and development tax credit (which, according to the latest data, has actually done little to stimulate private investment in research; see *Nature* 23 August, p.615) and for an easing of anti-trust laws to allow companies to collaborate on research.

The Democrats, who, as a rule, have tended to make public support for science *their* issue — albeit a minor one — find themselves at a loss for ammunition with which to launch an attack.

The Democratic platform calls among other things for an unspecified increase in "commercially rated" research and development, apparently a reference to the one area in which the Reagan Administration has cut back applied research. It also includes some vague language about establishing a programme to train scientists and engineers modelled on the Land Grant Act that created the state agricultural colleges and continues to provide them with an automatic annual handout; and some much vaguer language about fostering cooperation between academic and industrial researchers.

The problem that the Democrats and their nominee Walter Mondale face is that the Reagan Administration has actually been very good to science. After faltering the first year (cutting the National Science Foundation budget and sending shivers down the spines of scientists who feared that David Stockman's axe would be turned on them), Reagan has been unfailingly generous to research — basic research, that is.

Federal support for basic research was running at \$5,000 million a year in 1981 when Reagan became President; his proposed budget for 1985 provides for \$7,900 million. The National Science Foundation has fared particularly well, receiving a 17 per cent increase last year and a 15 per cent increase this year.

These gains for basic research have however, come at the expense of civilian applied research — overall, civilian

research and development has not grown under Reagan. The major victims have been the large energy demonstration and engineering projects that the Carter Administration favoured. The absolute gains have been reserved for defence, which has seen an enormous growth in its research and development budget, from \$16,500 million in 1981 to the \$33,900 million proposed for 1985. Still, by refocusing civilian spending on basic research, Reagan has apparently neutralized those in the research community who might otherwise be complaining that civilian research and development has been sacrificed for a defence build-up.

Dr Frank Press, science adviser to President Carter and now president of the National Academy of Sciences, agrees that science is not going to be an issue this year: "Both candidates are going to be extremely supportive of science", he says.

Mondale has picked up on several related issues that may show greater sympathy with the scientific community, however. In addition to pledging a real

growth of at least 3 per cent per year for civilian research and development and a \$4,500 million fund for a five-year modernization of university laboratories and research libraries, Mondale has in several recent statements rejected the Reagan Administration's efforts to clamp down on scientific communication.

Mondale says that the administration, "in its exuberance to restrict technical products and information to the East", has hurt the competitive position of US companies seeking to trade in the West. And he has called for the minimum controls on open scientific discussion necessary for national security. Although the Department of Defense (DoD) recently dropped plans to restrict publication of "sensitive", though unclassified, research — plans that were strongly opposed by the research universities — the administration is continuing to close certain scientific meetings under DoD sponsorship to non-US nationals; and Washington representatives of the universities are convinced that a second Reagan term will bring about a redoubling of similar efforts to clamp down on the free flow of information.

And of course sharp differences between the candidates emerge on science-in-society issues: space weapons, arms control, federal support for elementary and secondary education and environmental regulation.

Stephen Budiansky

Databases

Limited access to BIONET

THE rules for allowing access to the accommodation of nucleic acid sequence data sponsored by the US National Institutes of Health (NIH) have now been made public by Dr Joshua Lederberg, president of the Rockefeller University, New York, and chairman of the National Advisory Committee of BIONET.

The database consists chiefly of two streams — data accumulated by the NIH project, which is managed by Intelligenetics Inc. and Los Alamos Scientific Laboratory, and data accumulating on the parallel database at the European Molecular Biology Laboratory in Heidelberg, West Germany.

According to a statement by the BIONET committee, access to the NIH database will be open without charge to users at government and non-profit establishments, but access by overseas users and scientific investigators working for commercial organizations will be considered on a case by case basis by the advisory committee.

The plan is that applications for access (on a special form) will be accepted from qualified applicants until the system is full, "at which time... steps will be taken to remedy the situation". The database can be reached through the CompuServe data

network, which links the database with most major cities in the United States. Users will have access not merely to nucleotide sequences but to libraries of computer programs developed for analysis and other purposes.

As well as ordinary users of the data, applications will also be considered from people whose chief interest is collaboration in the development of the databank, chiefly through the design of computer programs.

Just now, it seems, the Heidelberg databank is something like one million nucleotides behind the accumulation in the United States, chiefly because of the need to incorporate a mass of material recently obtained from the NIH system. According to Dr G. Hamm, in charge of the Heidelberg database, these are still early days in the use of sequence data — even though more than 2.5 million nucleotides are included in the store at present. By his account, while most investigators still use the database as a means of comparing new sequences with those already known, and for searching for common elements in a variety of genetic data, there is increasingly an interest in using the database as a means of identifying interesting problems still to be solved. □

US space research

Moon beckons for NASA

La Jolla, California

AFTER 15 years of coasting along with ill-defined goals, the National Aeronautics and Space Administration (NASA) is setting its sights on a future which may include a return to the Moon.

Since President Reagan's State of the Union speech in January — when he approved the idea of a civilian space station and proposed his "star wars" defence — the agency has sponsored three study groups which asked "What should we do after the space station is built in 1992?"

The first met at Los Alamos in April. Called the Lunar Base Working Group, the 40 space experts in attendance concluded that the United States should return to the Moon. Their report to NASA, which will be released at a press conference in Washington, DC, in October, includes a list of scientific experiments that could be conducted on a lunar base. These include a radio telescope on the backside of the Moon which would not suffer electromagnetic interference from Earth and a laboratory for exploiting the Moon's vacuum.

The second, working under the rubric of a "Technological Springboard to the 21st Century", met for ten weeks at the California Space Institute in La Jolla. The 20 participants made their recommendations to NASA last week. It "makes sense", said the study's leader David McKay, a geologist at Houston's Johnson Space Center, for the space agency to consider using lunar materials for carrying out its future space missions. Whenever pioneers have ventured onto new territories, Dr McKay said, the first thing they do is use local materials.

If astronauts go to the Moon after a space station is finished, the study group said, they will inevitably start to become more self-sufficient and to rely less on communication with the Earth. Thus it is not too early to think about planning a space programme that relies on resources found in space.

The group, half of whose members had no previous association with NASA, was not, however, unanimous in this conclusion.

Proponents of the space resources scenario foresee the Moon and the asteroids as "gas stations" in the sky. The Moon, they note, is half oxygen which can be extracted and liquefied. The asteroids are rich in water. Thus rocket propellant — liquid hydrogen and liquid oxygen — is there for the taking. Unprocessed regolith can be thermally sintered for bricks, tiles and pipes. Carbonyl chemical processing could turn out iron alloys. A solar furnace could cook up glass, ceramics, metals and three kinds of cement.

Critics of this scenario rested their case on economics. "A lunar base is a dead-end", said Rocco Pazzolare of the Univer-

sity of Arizona. "It's silly to put Manhattan on the Moon", said William Lewis of Clemson University.

The Earth is a water planet, Dr Fazzolare said. It is abundant in hydrogen and oxygen. The problem, he said, is not to figure out how to mine the Moon but how to get resources out of the Earth's gravity for less cost. Thus, he and others proposed that the American space effort should focus on "Big Dumb Boosters" to lift more material into orbit more cheaply.

The third group is working within NASA to analyse the many suggestions coming its way. The direction the space programme

will take after the space station, they note, will affect how the station should be built. As the station is now on the drawing board, planners want to know what comes next.

At the La Jolla meeting, there were three reasons given for US space efforts — fear, greed and curiosity. The fear people said the Soviet Union might get ahead of the United States and that is reason enough to go. An expert on space law pointed out that American interpretation of space treaties says that whoever gets to the Moon first will have the biggest say in how it is used. The greed people said continued commercialization is inevitable and that it ought to drive NASA's engines. Information is already big business and materials processing is soon to follow.

Sandra Blakeslee

Small space platforms favoured

Washington

A SCIENTIFIC advisory panel has strongly urged the National Aeronautics and Space Administration (NASA) to drop plans for including two large orbiting platforms as part of the design of the proposed space station (see above). The Task Force on Scientific Uses of Space Station recommended instead that NASA should develop a fleet of small platforms that could be tailored to the needs of individual experiments. Separate platforms could be pointed in different directions and would avoid the problems of interference and vibrations caused by combining many experiments on a single large platform.

The task force's recommendations come just one month before NASA is due to issue its request for proposals for an initial design and definition study for the space station. Dr Burton Edelson, associate administrator of NASA for space science and applications, assured the task force that its views would be taken seriously.

NASA is known, however, to favour the concept of two large platforms; John Hodge of the space station programme told the task force that the large platforms

would offer a more "unified" approach to the station programme and would also be well suited to commercial projects, such as materials processing in space. One idea is to have one of the platforms orbit along with the station while the second travels in a polar orbit.

The official estimate of the cost of the space station is \$8,000 million. Task force members have suggested, however, that if the cost of scientific instrumentation and of launching the station are included, the true figure approaches \$18,000 million. Congress has so far appropriated only \$155 million, for the initial design work.

Other recommendations from the scientific task force include the construction of a "garage" within the station that could be used to assemble and repair satellites; construction of a separate module in the station for animal housing for experiments; and expanding to 10 the proposed 6-8 man capacity of the station to allow for six scientists.

The chairman of the task force is Dr Peter Brinks of Stanford's electrical engineering department.

Stephen Budiansky

Voodoo on the campus

Washington

FOR those who have forgotten that medicine is a young science, the University of Virginia is sponsoring a seminar this week featuring Dr Reinhold Voll, a German physician and the inventor of "electrodermal diagnosis" (EDD). According to Dr Voll, EDD can detect "chronic and terminal disease" by measuring skin resistance. A device, called the EAV Dermatron, is used to probe one of 850 acupuncture points on the skin that correspond to various internal organs, according to Voll, who has based his technique on traditional Chinese medicine.

The seminar is being held by the medical school's Health Information Center, which, in announcing the seminar, felt compelled to include a modest disclaimer:

"Admittedly, some of Dr Voll's claims sound rather speculative to Western scientific thought, such as the assertion of a direct relationship between acupuncture points and specific anatomical structures and physiological body functions, or EDD's ability to test the efficacy and tolerance of medication prior to its prescription. But then wasn't it scientific speculation which led to the development of the telephone, light bulb, television, laser and transistor, to name but a few inventions in our time?"

No double-blind trials have yet been carried out on the technique. Physicians attending the seminar are, however, encouraged to bring along patients with "well established or uncertain diagnosis" to test it out.

Stephen Budiansky

Soviet education

What prospects for reform?

NEXT Saturday, 1 September, the Soviet Union will celebrate its new "Day of Knowledge". Although the opening of the academic year has long been observed with special feature articles in the Soviet media, the raising of the event to the status of a nationwide festival is associated with the reform of primary and secondary education which comes into force next month.

The new reform, aimed at bringing up young people to meet the challenge of the "scientific and technological revolution", envisages three major changes. Compulsory schooling is to begin a year earlier — at six years of age rather than seven in most cases, and at five where, as in Estonia, the difficulties of the local language are considered to need an extra year's schooling. There is to be a "resolute improvement" of "military patriotic" education, preparing boys for their army service and girls for civil defence work. Most important, the "Leninist principle of a unified labour and polytechnical school" is to be implemented so that all pupils from senior forms are involved in "labour training" in appropriate production enterprises.

Of these three points, the upgrading of "military-patriotic education" seems to have crept into the resolution at a fairly late stage. (It was not mentioned, for example, in *Pravda's* account of the Central Committee meeting on 11 April which discussed the resolution in otherwise considerable detail.) The lowering of the school age is apparently necessary to allow the pupil to absorb new scientific knowledge, as well as providing more time for labour-training and "military-patriotic" work. The labour training itself, however, has an air of *déjà vu*. Superficially it looks like a return to the Khrushchev era, when an over-zealous attempt to move classes from school-room to factory and *kholkos* provided managers with a ready-made excuse for missed targets — that the children's presence had interfered with production schedules!

Part of the trouble with the Khrushchev scheme, it appears, was that nobody seemed quite clear whether the work training was meant to prepare the pupils for a job, or was simply an ethical lesson in the value and dignity of labour. This time, however, "labour training" is to be part of an improved programme of "vocational guidance", in which a production enterprise, organization or institution will be assigned to each school, and must allocate to the children "equipment, workplaces, skilled cadres and raw and semi-finished materials".

Just how this assignment scheme will work is not easy to see. Leading party activists have reiterated that the aim of the reform is to turn out "polytechnically" trained young people, who can respond rapidly to technological innovation

without requiring major retraining. Assigning a factory to a school, however, suggests that the opportunities for vocational training afforded to the latter's pupils will be limited. On the other hand, taking the children to the factory for an afternoon or so each week will not solve the problem of technical training facilities in schools, many of which, to judge from complaints in the Soviet media, are under-financed, ill-equipped and often housed in makeshift and unsuitable premises.

The reform appears to have caught the educational profession somewhat unprepared. During the "public debate" which preceded the formal resolution, numerous class and head-teachers pointed out that the reform would demand new

textbooks and syllabuses, and also the training or retraining of additional staff. This would in its turn require a replanning of school budgets — no easy matter in the middle of a five-year plan. Pedagogic colleges, in particular, were perturbed that they would have hurriedly to revise their teacher-training courses.

Already, it appears, some doubts are being voiced as to how rapidly and effectively the new reforms can be carried through. A meeting of the Politburo on 1 August noted that "not all industrial ministries and departments" had "genuinely" started to cooperate with the scheme. An All-Union teachers' conference in Moscow last month noted that under the present training scheme not more than a quarter of school leavers use the labour skills they have acquired, with the implication that things may not be very different under the reform. **Vera Rich**

Japanese space research

Halley probe on show

Tokyo

THE construction of Planet-A, Japan's Halley's comet probe, has just been completed and the satellite is now on display at the new Sagami Research Centre of the Institute of Space and Astronautical Science (ISAS), near Tokyo.

Considerable excitement surrounds the completion of Planet-A, for it and the MST5 test satellite which will be launched before it will be the first satellites Japan has ever sent into deep space. ISAS, around which Japan's independent, university-run space programme is centred, has always managed to keep launching scientific satellites on a small budget, and Planet-A has been cleverly designed to do as much science as possible while keeping payload weight to the minimum. Altogether the satellite will weigh just 138 kg and carry 12 kg of scientific instruments. The launch vehicle will be a specially improved version of the three stage solid-fuel Mu3S which has already been used to put several satellites into Earth orbit. Addition of two strap-on boosters to a slightly larger rocket will give enough power to put Planet-A into orbit around the Sun.

Planet-A will be launched around 15 August 1985, after a further round of tests, disassembly, reassembly and even more tests have been completed, and will fly within 200,000 km of Halley's comet on 8 March 1986. Its major aim is to take vacuum ultraviolet (Lyman alpha line) images of the hydrogen coma of Halley for ten days or so before and after perihelion. Close to the Sun, volatile materials — principally water — will sublimate from the comet and be photodissociated to form a large hydrogen coma around it. The images should help to explain both the processes of coma growth and decay and the importance of the various routes, involving formation of intermediate radicals, by

which hydrogen atoms are released from the water. To take images of the hydrogen coma, the camera lens and the charge-coupled device detectors should ideally be mounted on a non-spinning platform. But to avoid the weight of a complete 3-axis control system and differential heating of the satellite by the Sun, the satellite will be kept stably rotating at about 5 revolutions per minute (r.p.m.). While pictures are being taken, a momentum wheel will reduce spin down to about 0.2 r.p.m. and the image blur will be prevented by shifting the image on the charge coupled device in synchrony with the spin.

At the higher spin rates, a different set of instruments will measure the interaction of the solar wind with the comet's plasma tail and look out for turbulence produced in the tail and a coma by solar flare shock waves. This will be achieved by charged particle detectors for electrons and ions designed to measure the distribution of the solar wind plasma within ± 30 degrees of the ecliptic plane as the satellite cruises towards the comet.

During this phase, simultaneous measurements of the solar wind from as many probes as possible are desirable, and to help achieve this, clever use is to be made of the MST5 test satellite.

MST5 is to be launched in just four months' time — on 5 January 1985 — and is principally designed to test the new launch vehicle, attitude and control systems and the communications link. But it will also carry plasma wave probes and instruments to measure solar wind ion density and bulk velocity, and the interplanetary magnetic field. After launch, it will circle the Sun and then return to within 0.1 astronomical units of both Halley and Planet-A in March 1986 to complement Planet-A's solar wind measurements. **Alun Anderson**

Uranium shipment

Soviet contract nobody wants

THE French ship *Mont-Louis*, sunk in 60 feet of water in the world's busiest seaway, the English Channel, with a 250-ton cargo of uranium hexafluoride bound for the Soviet Union, is an embarrassment to Europe — not just for environmental reasons (and the danger may anyway be much less than has been claimed) but because nobody in Europe wants to carry on with the roughly thrice-a-year trade that the *Mont-Louis* was plying.

"We tried to cancel the contract last year", says Jean-Claude Magnac, secretary-general of the French nuclear fuel cycle company, COGEMA, "but the Russians wouldn't have it".

The origin of the agreement in 1973 was that Europe seemed set for a vast pressurized water reactor (PWR) construction programme. PWRs need slightly enriched uranium (2-3 per cent) and Europe at that time had no major enrichment capacity. A facility, called Eurodif, was planned, but environmental opposition was so great that it was feared it might be impossible to construct, says Magnac. So France, Belgium, the Netherlands and West Germany signed a deal with the Soviet Union to enrich uranium that they would supply (as hexafluoride).

The deal was for the Soviet Union to provide a certain amount of enrichment (measured in units called "swus", for "separation work units") over the period 1973-85. But then came the embarrassment: Eurodif was constructed after all, while France and to a lesser extent Belgium were the only countries to go ahead with a large PWR programme. According to a spokesman for the Belgian utilities, "we just don't need the Russian swus now".

But the Soviet Union, during renegotiation last year, insisted on fulfilling the original contract, to provide a certain total of swus. Since that seemed unlikely to be reached by the 1985 end-date envisaged in 1973, this entailed actually extending the contract at a lower rate of supply — to 1995, said the Belgian spokesman, until the original swu total is reached. Meanwhile, Eurodif is working well under capacity, though less because of the Soviet supply than the shortage of European PWRs. Belgian reactors, for example, have a demand for 650,000 swus a year, while the Soviet supply is less than a tenth of that.

According to Magnac, the supply to France is also a small proportion of French requirements. None of the trade involves enrichment to military grade levels, he says, so there is no conflict with the nuclear non-proliferation treaty, to which the Soviet Union (but not France) is signatory. It is simply a commercial nonsense to which Europe is tied by a business contract.

Robert Walgate

Medical education

New "private" school to open

BRITAIN's first independent medical school will become a reality in September, after delays caused by problems in finding a suitable site and, more seriously, a lack of funds (see *Nature* 304, 576; 1983). The Hunter School of Medicine, which will admit its first students in January 1985, is a non-profit making body with the status of a registered charity. It will occupy the premises in London occupied until now by Bedford College, which has merged with Royal Holloway College, another college of the University of London. The Hunter School is to share the premises with Rockford College of Ohio in the United States.



Supporters of the project feel that there are fundamental faults in the present method of selection and training of medical students that are unlikely to be rectified within the present teaching system. Because, they argue, universities admit students solely on the basis of academic standing, well balanced, broadly educated students able to communicate easily and sympathetically with their patients are "selected out" in favour of those more interested in medicine as a purely scientific discipline. Furthermore, courses are too information-oriented and, in wholeheartedly embracing the new high-technology medicine, have paid too little attention to the human aspect of doctor-patient relations.

The new medical school will charge its students (30 British and 20 foreign each year) £7,500 in annual fees. This income will provide for the day-to-day running of the school while capital funds will come from charitable sources. A public appeal is to be launched to raise £5 million to buy buildings and provide scholarships and bursaries and there will be a student loan scheme.

The Hunter School will attempt to select its students on the basis of a range of desired qualities including adaptability, personality, humour, a willingness to take on responsibility, a genuine interest in medicine and, of course, the ability to carry through an arduous programme of medical training. It is hoped that, by accepting only

students over twenty-one who have completed some form of training since school, the task of selection will be made easier.

The curriculum will involve the teaching of computer science, information technology, ethics, psychology, medical administration, community medicine and other subjects commonly considered of secondary importance. The Hunter School takes the view that proficiency in these areas is vital to doctors working in sophisticated modern hospitals.

As a new medical school (the first in London for a century), the Hunter School will need to have its courses approved by the General Medical Council, but it is hoped that by the time the first graduates emerge into the outside world, the qualifications conferred by the school will be recognized. There is however, a European Community directive requiring medical teaching facilities to be supervised by a university, but Professor C.A. Pasternak of the Hunter School's board of management, an advisory body, is confident that this should pose no serious problem since use will be made by the Hunter School of facilities at other teaching hospitals connected with universities.

Marcus Chown

UK medical research

Funds scarce

THE UK Medical Research Council (MRC), in common with its counterpart the Science and Engineering Research Council, is now in the distressing position of having to refuse funds for research judged by peer review to be of the highest merit.

Applications for research grants are graded by the grant awarding bodies with an alpha, beta or gamma. Alpha research proposals are those to be supported "at all costs" and gamma denotes a proposal to be rejected outright.

MRC figures show that the percentage of alpha money approved (for research programmes of three and five years' duration) for the 1983-84 award session has dropped to just 53 per cent from the previous year's level of 87 per cent. The money requested for alpha research proposals has risen in the intervening year from £48.7 million to £58.6 million, but only £31.4 million has been awarded in 1983-84 against £42.3 million the year before.

In 1982-83, over 20 per cent of those applications rated alpha by the Science and Engineering Research Council (see *Nature* 26 July, p.267) were not supported whereas no alphas went without funds and many betas also received money until 1978-79.

Marcus Chown

Polish geology

Government policy attacked

A LEADING Polish geologist has called for a rethinking of the government's attitude to one of Poland's major natural resources — sulphur. Interviewed in the party daily, *Trybuna Ludu*, Dr Stanislaw Pawlowski, a member of the Polish Academy of Sciences, urged the need for legislation to govern the exploitation of mineral resources, and criticized current attitudes on the development of mining areas.



Polish sulphur mine

During the past few decades, he said, "a great number of irreversible errors have been made".

Poland, according to the nineteenth century "father of Polish geology", Stanislaw Staszic, is "built on sulphur" but the vast reserves that form the basis of the modern sulphur industry were not found until the 1950s, although preliminary surveys started in 1937, and the resulting field data were secretly processed during the Second World War under Nazi occupation.

Ostensibly, the *Trybuna Ludu* interview deals with Pawlowski's role in the discovery of sulphur along the Zawichost-Kurdwanów dislocation along the Vistula river in the region of Sandomierz and Tarnobrzeg. Pawlowski, however, is concerned not so much with his discovery in 1953 of vast sulphur beds at depths of as little as 15m but rather the bureaucratic difficulties which followed.

Before the new sulphur beds were discovered, a project had been floated for the construction of a sulphuric acid works at Gackie near Buska-Zdroj, using gypsum as the raw material. Although this process at least three times as expensive as the production of sulphuric acid from native sulphur, Pawlowski had a hard fight to get the decision-makers to change their minds, principally because they feared that the new beds would prove insufficient. Since then, the mood has changed and the abundance of sulphur has led to a somewhat careless attitude. The Basznia mine near Lubaczów, with an annual output of a mere 20,000 tonnes, is officially considered to be of little importance (wrongly so, says Pawlowski) although a number of mines in the United States have outputs of this order.

More seriously, inefficient mining is causing the irretrievable loss of usable

sulphur. The organization of mining work is far from satisfactory, says Pawlowski, and many tonnes of workable sulphur are being left in the ground to be lost forever because "tectonic, hydrological and other conditions are changing".

Poland uses two methods of sulphur extraction. In the deep deposits at Grzybów and Jeziórko, a modern underground liquefaction process, using super-heated water, is employed, yielding a high-quality product. The shallow beds discovered by Pawlowski, however, are worked by opencast techniques. Apologists for the latter method have always stressed that operations are carried out with due regard for the environment, but in the sulphur town of Tarnobrzeg, for example, the trees have a sickly yellowish tinge and the air has a strong sulphurous

smell. Pawlowski attacks the whole concept of the planning of Tarnobrzeg, which, he says, has been allowed to expand towards the mining area and is now surrounded on three sides, by mine-workings.

Pawlowski's radical solution would be to give the town "a window on the west" by building a bridge across the Vistula, and constructing a new town on the west bank where the inhabitants could enjoy "clean air, fresh water and uncontaminated food". At the same time, Pawlowski urges new environmental controls in sulphur-mining areas, including the designation of green belts. Geologists working on the sulphur beds of the Osieka-Baranów region have, says Pawlowski, warned of the pollution threat inherent in working the deposits. As far as he knows, however, no action has so far been taken, even though Baranów Castle, an important historical monument, would be directly threatened.

Vera Rich

Palaeontology

Hungarian flint-mine found

Budapest

THE discovery of a palaeolithic flint-mine at Farkasrét (Wolves' Field) on the outskirts of Budapest in Hungary seems likely to generate radical rethinking about the technology of the Mousterian era (50,000 BP). Although flint-mines and ateliers of this period are fairly common in Europe, the Budapest find includes not only flint shards left after the shaping of tools but also mining implements made from deer antlers. Similar picks and hammers have previously been found, but only from the Neolithic era. Indeed, since the form of the antlers imposes severe constraints on the design of such picks and hammers, Dr Veronica Gabori-Csank of the Budapest Historical Museum, who found them, assumed that what she was investigating was a neolithic deposit. Only on 21 June, the thirtieth day of the dig, did she discover among the implements a typical Mousterian hand-axe, apparently discarded by the artificer because of a flaw in the stone. Since then, other Mousterian flint artefacts have come to light, convincing Dr Gabori-Csank that the mining implements date from some 50,000 BP, from a temperate period before the Würmian glaciation.

The Farkasrét site has been known as a potential source of prehistoric material since 1969, when a deer-antler mining tool was discovered by a university student, but he neglected to mark the exact site, noting only that it came from a cliff alongside a suburban road. This lack of precision, and the somewhat atypical nature of his find meant, however, that the site was neglected until early this year, when another implement was found in the exposed face of the cliff, and excavations were started.

Examination of the exposed cliff face revealed a cross-section of a former gully, now completely filled by detritus. Working in only a small section of this gully, Dr Gabori-Csank has so far discovered fifty-eight magnificent specimens of mining tools, which are now undergoing conservation treatment. The high quality of the specimens, however, has raised problems with carbon-14 dating. Although the Nuclear Research Institute of the Hungarian Academy of Sciences at Debrecen would seem the obvious place for such tests, the outmoded equipment there would require the destruction of some 1.5 kg of material. This Dr Gabori-Csank would be reluctant to sanction, particularly since the Mousterian finds provide strong *prima facie* evidence of the palaeolithic origin of the tools. Carbon-14 dating will therefore have to wait until the coming winter, when a slot has been reserved for it at a unit in Hanover, which will require the sacrifice of only a few grams.

Hungary may be short of modern equipment, but it is not short of enthusiasm. The new rector of Budapest's Eotvos Lorand University, Dr Jozsef Fülöp, is a geologist with a special interest in prehistoric flint-mines.

In May, while still in his former post as president of the Central Geological Office, he arranged for the funding of a geophysical survey of the site, which traced the course of the gully for future excavations. In 1986, moreover, Budapest will host an international archaeological conference on flint-mines, already arranged before the Farkasrét discoveries. It is now hoped that an on-site museum will be constructed next year and inaugurated during the 1986 conference.

Vera Rich

East-West relations

SIR — That the comments on the deterioration of East-West cooperation in science (*Nature*, 28 June, p.735) came from a political commentator of the Novosti Press Agency, Alexander Sapsai (9 August, p.446), and not, as one might have expected, from a Soviet scientist, is not difficult to explain. All issues of *Nature* which discuss the problems of Soviet science do not reach the libraries in Soviet research institutes, or the Central State Lenin Library in Moscow, or libraries of the Academy of Medical Sciences of the USSR or any other specialized or republican academy in the Soviet Union. More than half of the issues of *Nature* for each year are not listed in the libraries' catalogues, but are kept in "special holdings" where they can be read only by a few scientists with special clearance.

If Alexander Sapsai thinks that it is only US policy which is to blame for poor cooperation in science between the East and the West, I would like him to explain a few disproportions. There were about 2,000 Western biochemists at the 16th FEBS Meeting in Moscow last June, 75 of them from Great Britain. However, there was not a single Soviet biochemist at the 13th FEBS Meeting in Israel in 1980. Certainly the Soviet officials consider Israel as a special case — but these are political reasons, that have nothing in common with the interests of science. There were about 1,000 scientists from the West at the 9th International Gerontological Congress at Kiev in 1972. However, at the 12th International Gerontological Congress in Hamburg in 1981, there was only one Soviet scientist, Professor Dmitry Chebotarev, who is director of the Kiev Institute of Gerontology. All other Soviet gerontologists who applied to participate and were in the programme were unable to attend.

These disproportions I witnessed personally, and I could give many more examples. But that is not necessary. Every Western scientist knows how unpredictable and unreliable is Soviet participation in international congresses, conferences and symposia organized outside the Soviet Union, even in East European countries such as Hungary, Romania, East Germany and Poland. At the recent European Congress of Gerontology in Budapest (1–4 September 1983), only a few Soviet gerontologists were able to take part, fewer than from Poland, Czechoslovakia or Denmark, not to mention France or Britain.

The examples which Alexander Sapsai quotes in support of the Soviet Union's positive attitude to international cooperation in science and technology all involve high-level members of the scientific elite. At the level of younger scientists, the existing restrictions have been introduced by the Soviet side, more recently and

irrationally by legislation. A special decree in August 1982 severely restricted the scientific and other literature which could be sent abroad from the Soviet Union; the postal services were forbidden to accept books for mailing abroad without special permission. A second decree, passed in May 1984, made punishable most unauthorized contacts between Soviet citizens and foreign visitors.

The Andrei Sakharov case makes it absolutely clear that the Soviet Government can keep a prominent scientist silent and incommunicado, and can completely ignore the opinion of the world's scientific community on the matter. Alexander Sapsai was proud to mention that the Soviet Union has 1.5 million research scientists, "25 per cent of the world figure". But how many of those 1.5 million have ever met a foreign colleague, inside the Soviet Union or outside? I once tried to study the real level of Soviet scientific cooperation with scientists in other countries at the level of rank and file young scientists¹. I found that the Soviet record was better than that of only two other countries — Albania and North Korea. ZHORESA MEDVEDEV

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1. Medvedev, Zh.A. *Soviet Science*, (Oxford University Press, 1979).

Science in India

SIR — The trouble with science in India is that it lacks a base. It is not possible to develop science when the whole educational system from the primary school to the university is concerned merely with learning by rote to secure marks in order to pass an examination and secure a job.

Science in India at best satisfies the intellectual curiosity of a small elite, which is itself divorced from the reality of the country and its problems, and which looks to the West for cultural inspiration and kudos. For the vast majority, science is a means of earning a living. In a highly competitive society, this means obtaining the highest possible paper qualification. This explains why India has the third largest number of scientists yet such a meagre scientific output.

The scientific elite also ends up being frustrated because, having been trained in Western countries where the scientific culture is an integral part of society, it finds that very little can be achieved in a country where Western science is not part of the culture. This results in time being wasted in overcoming petty bureaucratic hurdles and delays, by all scientists from those in the Department of Science and Technology to the local head of an institution or depart-

ment and the support and maintenance staff.

A good scientist therefore eventually gives up the struggle and emigrates to the West if the opportunity arises. Failing that, he or she joins the majority whose ambition is to use science to better their social and financial status. These are the future directors and heads of scientific institutes and departments in both the public and the private sector. For them, it is politics and not science that is the path to success. They lend themselves to being manipulated by politicians, bureaucrats and their own seniors. Not much can grow under the shadow of such banyan trees. A hierarchical approach cannot stimulate the young; it only encourages a subservient atmosphere that stifles independence and originality.

It is therefore not surprising that the achievements of Indian science fall far short of what might be expected from the substantial inputs in manpower and finance. There is no shortage of problems for scientific investigation, nor is there anything wrong with the intelligence and originality of our young scientists, for they mature admirably in the West. It is time that India realized that the problem lies not with our scientists but with the infertile soil we have created in which no worthwhile science can grow. N. H. ANTIA

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Miracles

SIR — Dr Sam Berry and others have not invited trouble as surely as the author of the leading article "Miracles do not happen" in *Nature* (19 July, p.171) who takes as his title a statement that Berry *et al.* have shown to be logically unjustified.

As a scientist he can only say, as he rightly does, that since science operates by generalizations of our experience, miracles are by definition excluded from its sphere of enquiry. He cannot therefore logically conclude that they do not occur: this is a statement of faith, or a personal opinion based on atheistic belief. The claim that "miracles do not occur" is as unjustified in a scientific context as the claim that "evolution does not occur" is in a theological context.

I believe that evolution has occurred — this is a scientific belief based on appropriate evidence, and the subject is outside the bounds of proper theological study.

I believe that miracles have occurred — this is a religious belief based on appropriate evidence, and the subject is outside the bounds of proper scientific enquiry.

Overstepping the bounds of science to argue against miracles is in my view as mistaken as unduly extending biblical authority to support arguments against evolution. JEREMY H. MARSHALL

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Continuing doubt on gravitation

The latest measurement of the gravitational constant does not improve the precision with which this important quantity is known. Is there a more radical approach?

EMBARRASSMENT at the difficulty of obtaining an accurate value of the gravitational constant (G) has long since ceased to be a joke. So much is clear from A.H. Cook's latest attempt at a measurement of G , now published (Y.T. Chen, A.H. Cook and A.J.F. Metherell, *Proc. R. Soc. A* **394**, 47-68; 1984). Part of the joke is that Cook and his colleagues at the Cavendish Laboratory, Cambridge, set out to measure not G , the constant in the inverse-square law of gravitational force, but departures from Newton's law of gravitation. Their conclusion is that the inverse-square law holds even more precisely than had been thought, but their implied value of G is hardly more accurate than that of their predecessors in the measurement of this most elusive constant.

The position is quite tantalizing. Nowadays the strength of gravitational forces in the neighbourhood of substantial masses such as the Sun or the Earth can be estimated with increasing accuracy. Measurement of Earth-satellite orbits, for example, can now yield the value of GM (where M is the mass of the Earth) to within one part in 10^7 , essentially the precision of its distance measurements. But the value of G remains obdurately uncertain to the tune of one part in 10^5 . The consequences include enormous absolute uncertainties in, say, the mass of the Earth (see *Nature* **302**, 11; 1983).

None of this diminishes the cleverness of what Cook's team has been up to. In the trade, the measurement will be known as a torsion balance experiment, in the tradition begun by Cavendish at the end of the eighteenth century which has the advantage that timing self-oscillations obviates the need to know the mechanical properties of the dispersion. In this case, the beam of the torsion balance is a 60-cm aluminium tube suspended by a tungsten fibre nearly a metre long and $75\ \mu\text{m}$ in diameter: much of the effort underlying the experiment has gone into the annealing of this filament, in a process devised so as to eliminate dislocations from the crystal lattice and which occupies roughly one month for each fibre. The torsion balance itself has a spherical mass suspended from one end and is entirely enclosed in a vacuum vessel. Electric and magnetic fields are excluded by shielding.

Making measurements with this system is far from being child's play. The torsion balance is fitted with a 3-m optical lever consisting of a beam from an He-Ne laser

whose angular deflection, detected by a photocell, can be measured to a millionth of a degree. To ensure the stability (and integrity) of the balance, there is a pair of electrostatic plates at the blind end of the balance beam and an electronic servo-system so as to provide appropriate negative feedback.

Breaking with tradition, the Cambridge team has used as masses for deflecting the test-mass hanging from the balance beam neither spheres (Cavendish) nor annular rings (Long, 1977) but cylinders, arranged with their axes parallel to the balance beam. The calculation is that such an arrangement should be relatively insensitive to displacements in the accurate positioning of the cylindrical masses along their longitudinal axes.

Cook and his colleagues have been to some extent constrained in the design of their equipment by their underlying objective, to measure departures from the inverse-square law. For the past decade, there has been a train of speculation that if gravitational forces are ever to be explained as are the three other kinds of natural forces between material particles, by some mechanism entailing the mutual exchange of field particles of some kind, there will be a small exponential correction to the inverse-square law at sufficiently small distances. D.R. Long startled some of his colleagues nearly a decade ago (*Nature* **260**, 417; 1976) by claiming that there are indeed departures from the inverse-square law corresponding to a characteristic distance of 2 m. Detecting such variations from ideality evidently requires substantial separations between the gravitating masses. The benefit is that Long's characteristic distance has been pushed out to between 3 and 4 m. The snag is that the uncertainty of G implied by the measurements is greater than that of the most accurate measurement so far reported, that of G.G. Luther and W.R. Towler (*Phys. Rev. Lett.* **48**, 121; 1982) — in their torsion balance experiment.

Two general questions arise, of which the most immediate may be the difficulty of making substantially more accurate measurements. There are two long-standing obstacles to further refinement — the fact that gravitational forces are indeed much weaker than other kinds of forces, so that it is not possible to think of balancing gravitational and, say, electromagnetic forces in some clever electronic experiment, and the awkward circumstance that

the gravitational force between two masses depends on the product of the two. In measurements such as that of Cook's group, it is beneficial in some ways that test-mass hung from the balance beam need never be directly measured, but the consequence is that everything turns on the ratio of the masses of two external objects, which boils down to a problem in macroscopic metrology — and the assumption that the deflecting objects are homogeneous and identical in composition.

The time therefore seems ripe for a radically new approach to the design of measurements of G . The temptation to look for oscillating mechanical systems where masses might be made measurable by some resonance phenomenon should be resisted; the mass of the supports needed to keep an oscillating system stable would probably dominate the measurement. Levitating superconducting objects magnetically would have the disadvantage that they might be made into little dynamos. Bending beams of neutrons might do the trick if it were possible to measure velocity of momentum with sufficient accuracy.

Meanwhile, uncertainty about the correct value of G will persist. The internationally accepted CODATA value is $6.6720 \pm 0.0041 \times 10^{-11}\ \text{m}^3\ \text{kg}^{-1}\ \text{s}^{-2}$. George T. Gillies, at the Bureau International des Poids et Mesures, who has shouldered the task of keeping track of G and related measurements, points out that the three most recent values claimed to have superior accuracy (one part in 10^5) differ among themselves by more than any of the standard deviations claimed. It will not be easy to wring an agreed value from these measurements, or even to look for a precision much exceeding one part in 5,000.

According to Gillies' latest review of the position, completed in 1983, there is much less doubt about other kinds of measurements made of quantities related to G . From time to time, people have been concerned with questions as odd as the permeability of other materials for the gravitational force, the variation of gravitational force with condition of interacting masses and even the temperature variation. Most of the results are, of course, null results. There are signs of life only in the question whether G varies systematically with time, as first suggested by Dirac. The search for time variation G has recruited a small army of devotees, but that is another story. **John Maddox**

Molecular biology

No introns in insect globin genes

from Athel Cornish-Bowden

ANTOINE and Niessing report in this issue of *Nature* (p. 795) that the genes coding for globins in insects entirely lack the introns hitherto regarded as a stable feature of the globin gene family. In vertebrates, all of the known genes for myoglobin and haemoglobin are organized in the same way, with the coding regions interrupted by introns at positions that correspond exactly from gene to gene. Not only does this imply conservation of intron arrangement throughout the 600–800 Myr of vertebrate evolution, but the discovery (Jensen, E. O. *et al. Nature* 291, 677; 1981) that the leg-haemoglobin genes of leguminous plants contain three introns, two of them corresponding with the two seen in vertebrates, implies a much longer period of conservation.

Despite the embarrassing existence of plant leghaemoglobin, the globins have until now been regarded as characteristic of vertebrates. Nonetheless, the twelve different haemoglobins of the midge *Chironomus thummi thummi* are clearly true globins. Their amino acid sequences agree with that of the β -chain of vertebrate haemoglobin at about 16 per cent of sites, a degree of similarity that would arise by chance with a probability less than 0.01 per cent, and one that is not much less than the 19 per cent identity between human myoglobin and lamprey globin. A possible way to account for the lack of introns in insect haemoglobin genes is by arguing that they are not true genes coding for proteins in the normal living state but are 'processed pseudogenes' that are not expressed *in vivo*. Antoine and Niessing present experimental results, however, that would make this possibility highly unlikely.

The stability hitherto apparent in the organization of globin genes in vertebrates is not observed in other gene families. For example, human α_1 -antitrypsin and chicken ovalbumin are two proteins whose amino acid sequences show a clear relationship, but whose genes have completely different exon–intron arrangements (Leicht, M. *et al. Nature* 297, 655; 1982), which requires one to postulate either that introns have been inserted independently into the two lineages or that both have suffered deletion of introns from a common ancestral gene that contained at least ten. Current work by Nakanishi and co-workers (Tanaka, T., Ohkubo, H. & Nakanishi, S. *J. biol. Chem.* 259, 8063; 1984) on the gene for angiotensinogen, another protein from the same family, provides additional information: although rat angiotensinogen resembles α_1 -antitrypsin considerably less than α_1 -antitrypsin and ovalbumin resemble one another, its gene contains four introns

located at positions that agree closely with the arrangement in the α_1 -antitrypsin gene. This result indicates that intron arrangement is not maintained unchanged forever, and that modifications to the intron arrangement do not occur in step with the evolution of the corresponding proteins.

The lack of introns in the genes for insect haemoglobins is not difficult to accept unless one is committed to the hypothesis of stability of globin genes. Evidence from other gene families certainly allows one to postulate that an ancestral gene may have contained three (or more) introns, of which three remain in the plant genes, but only two in those of vertebrates and none in those of insects. Although, at first sight, it might seem surprising that vertebrates apparently correspond better to plants than to insects, the number of deletions postulated is extremely small in statistical terms, and if three deletions are placed at random on the evolutionary tree relating

plants, vertebrates and insects there is only about a 65 per cent likelihood that at least two of them will fall on the part of the tree connecting the plants to the point of separation between vertebrates and insects (assuming, for simplicity, that the vertebrate and insect lines separated about half as long ago as the plant and animal lines).

It may still be worthwhile to consider whether the leghaemoglobin gene might have been acquired by the leguminous plants much more recently than the evolutionary separation of the plant and animal kingdoms. The light-emitting bacterium *Photobacter leiognathi* carries a gene for a copper–zinc superoxide dismutase that is clearly fish-like, though different from the enzyme of its ponyfish host (Martin, J. P. & Fridovich, I. *J. biol. Chem.* 256, 6080; 1981); thus, transfer of genetic information from a eukaryote to a prokaryote appears to have occurred during the period of symbiotic association. In view of this precedent, it can hardly be regarded as impossible for the leguminous plants to have acquired their globin gene from an animal original. □

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Developmental biology

On to the vertebrates

from Julian Lewis

THE complexity of an embryo is the result of diversification and rearrangement: the tissues become subdivided into parts with different characters, and those parts move and become interwoven to form increasingly intricate assemblies. The body segments of a vertebrate provide a good example. As the neural tube is forming along the axis of the body, the long slab of mesoderm on either side of it begins to split up into a series of little blocks, the somites. Later, each somite in its turn is partitioned to give the dermis of the trunk, the cartilage and bone of the vertebrae and ribs, and the skeletal muscle cells. While these muscle and connective tissue cells are differentiating, other cells, with other origins, move in amongst them. Spinal nerves grow out from the neural tube and the spinal ganglia, while invading endothelial cells create a network of blood vessels. The nerves and vessels, in their own periodic arrangement, mirror the segmental pattern of the somite-derived tissues. Any such structure created by assembly of components from different sources begs the question, how is the assembly coordinated? Does the somite segmentation dictate the neural pattern, or vice versa, or does some common factor cause the tissues to segment independently in parallel? Experiments on chick embryos, described by Keynes and Stern on page 786 of this issue of *Nature*, give an answer to

this question, confirming the conclusions of Detwiler from his work on amphibians long ago (*J. exp. Zool.* 67, 395; 1934). The new findings at the same time throw light on another question that is deeper, subtler and more far-reaching in its implications: how is the physical pattern of segmentation in the mesoderm related to the pattern of intrinsic characters of mesodermal cells?

The methods used are simple: a whole-mount stain for early embryonic nerve fibres, and the traditional embryologist's technique of surgical rearrangement of the developing tissues. The first observation is that the nerve fibres always emerge from the neural tube at the level of the anterior (or cranial) half of each somite, and proceed to grow out through this half of the somite only. If a portion of the neural tube is excised, before any axons have emerged, and is replaced with its anteroposterior (cranio-caudal) axis reversed, the pattern of nerve outgrowth is the same: the axons still pass out through the anterior halves of the somites. Thus, the sites of nerve outgrowth are not determined by the neural tube, but by its surroundings. If, however, a portion of the somitic mesoderm is excised and replaced in reverse orientation at a similarly early stage, the nerve fibres emerge through the parts of the somites that are now posterior, but were anterior with respect to the original orientation of the mesoderm. Evidently the somites

impose a segmental structure on the nerves.

Neurobiologists will ask themselves the routine questions about factors controlling axon outgrowth; but the observations are more remarkable for what they tell us about somites and segmentation. It appears that the anterior and posterior halves of each somite, even though they look similar and give rise to the same range of types of tissue, are non-equivalent: in Jonathan Slack's phrase, the cells differ in their 'secret names'. Evidently, the nerve fibres can sniff these names out. Somehow, the mechanism that makes the anterior and posterior halves different must be coupled to the mechanism that splits up the mesoderm into somites. Conceivably, the cleft between one somite and the next forms as the result of a confrontation between cells of posterior character at the back of the one prospective somite and cells of anterior character at the front of the other.

The really topical speculation, however, must concern the parallel with insects. They too are segmented animals; and in them, too, it has been possible to show that each segment is subdivided into an anterior and a posterior compartment, containing cells that differ in their intrinsic character (Lawrence, P.A. *Cell* 26, 3; 1981; Kornberg, T. *Dev. Biol.* 86, 363; 1981). In the fruit fly *Drosophila*, as in the chick, the boundary between the anterior and the posterior portions is invisible to ordinary inspection, although it can be detected by the special techniques of clonal analysis. But the *Drosophila* work is far ahead in one crucial respect: a gene, named *engrailed*, whose expression marks the difference between the anterior and posterior cells, has been identified. Herein lies the excitement. So many of the genes controlling the formation of segments and the differences between segments in *Drosophila* have now been found and even cloned, that it seems we are at last on the verge of understanding how the DNA of an insect specifies its body plan; and if developmental biologists working on insects have an ultimate goal, that surely is it. The problem is no less central for vertebrates, but there we know practically nothing about the genes controlling pattern formation. As readers of these columns will have noticed, however, a DNA sequence common to several homoeotic gene complexes in *Drosophila* has recently been discovered, and it turns out to resemble sequences present in vertebrate genomes (Struhl, G. *Nature* 310, 10; 1984; Slack, J. *Nature* 310, 364; 1984). This makes it tempting to wonder whether vertebrates might after all be homologous to insects in the molecular biology of their segmentation and hence in their most basic developmental principles. This week's account of the paths of nerve fibres through somites in the chick may give a little extra encouragement to that speculation. □

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Solar physics

New lease on life for the Solar Maximum Mission

from E. L. Chupp

ON 24 April 1984 a major solar flare erupted on the Sun. Newly repaired and redeployed, the Solar Maximum Mission 2 (SMM 2) satellite was in a perfect position — pointing towards active region 4474 — to see it. Several of the instruments on board observed this flare, which was the largest seen by any SMM instrument since the satellite's launch in February 1980. That the flare was successfully recorded would not be an exceptional feat were it not for the fact that it occurred less than three weeks after repair, in space, of the satellite and of two of its instruments. Indeed, the astronauts, the Goddard Space Flight Center engineers and the men at Mission Control in Houston, Texas, turned 'almost failure' into success. The disaster of losing the satellite was at one point imminent but a computer program gave the Solar Maximum Mission an extended life. To appreciate the importance of this accomplishment, a review of the prospects for the renewed Solar Maximum Mission is in order.

In February 1980, SMM 1, a multi-modular spacecraft carrying seven experiments intended to observe the Sun, was launched from Cape Kennedy (*Sky and Telescope*, 199; September 1980). Equipped with a grapple-hook and a trunion pin, the satellite was designed for space shuttle retrieval. Moreover, the box-like units providing power, commands and attitude control of the spacecraft were replaceable, making feasible, for the first time, repair of faulty units in space. Although the primary purpose of these features was to create a retrievable and reusable spacecraft, when a pointing failure developed in the SMM Attitude Control System in November 1980, which meant that the four highly-directional telescopes studying ultraviolet and soft X-ray emission could not operate, plans to repair the satellite in space had to be put into operation. Three instruments continued observations until just before the repair took place. Repair was completed this April, and six of the seven instruments on board are again operational (*Sky and Telescope*, 498; June 1984).

Within three weeks of release of the SMM 2 satellite, bursts of solar X-rays were recorded by the hard X-ray burst spectrometer (HXRBS), which detects emissions from anywhere on the Sun. Since SMM 2 was not pointing its precision telescopes at the likely sources of these bursts, it was reoriented to point at a good prospect, solar active region 4474. Within minutes, at 2359 UT on 24 April 1984, a solar flare erupted, the most intense flare observed by SMM and possibly the most powerful ever recorded. The SMM γ -ray

spectrometer (GRS), which records the highest-energy emissions, measured γ -ray line fluences (time-integrated fluxes) at 0.511 MeV and 2.223 MeV, from nuclear processes in the flare, which were >400 and >600 photons cm^{-2} respectively. A fine pointed telescope measuring soft X-rays, the X-ray polychromator (XRP), was able to localize accurately the source of the flare emissions and detect flare X-ray lines, broadened by turbulence early in the flare. With its sophisticated set of instruments, the SMM 2 satellite is set to collect data that will lead to a new understanding of solar flares.

The prospects for addressing other significant issues of solar physics with the repaired SMM are excellent. The major scientific goal of SMM 1 was to perform coordinated observations of solar flares with all seven instruments. This required that four highly-focused telescopes, recording optical, UV and X-ray emissions, be pointed in advance at the active region that might produce the flare. Near the period of maximum sunspot number in 1980, there were many potential locations for flares, and as there are no methods available to predict where the largest flare will occur, the four pointing telescopes frequently missed the largest flares seen by the HXRBS and GRS instruments, which observe all flares visible on the solar disc. This was a serious concern because coordinated observations are essential to answer such questions as, how and when will a flare occur and, ultimately, how are the highest-energy emissions produced. Because there are fewer sunspots now, in the declining phase of solar activity, SMM scientists have a much better chance of pointing the spacecraft at the best flare site. On SMM 1, the hard X-ray imaging spectrometer (HXIS) was the first to sense an X-ray brightening, and gave a 'flare alarm' to the other instruments which then homed in on the flaring region and conducted a planned sequence of rapid-fire observations on a smaller field-of-view than in nonflare observations. As the HXIS instrument could not be repaired, the XRP will now provide the 'flare alarm'.

Several new approaches to studying solar activity are possible on SMM 2. A particularly intriguing issue is whether the nature of solar flare emissions changes during the solar cycle. Three SMM instruments have been observing the Sun nearly continuously since February 1980, and have collected the largest data base on the Sun from any observatory in space. In the first three years of the SMM 1 operation, the HXRBS, which does not require

precision-pointing, recorded almost 7,000 flares with photon emissions > 25 keV, most of which produced X-ray bursts in an uneven spiked manner. Some of the bursts, which take on a slowly varying character after 1 minute, seem now to be more frequent than in 1980.

Observations of the detailed timing of flare X-ray (XRP, HXRBS), γ -ray (GRS) and ultraviolet (UVSP) emissions will give insight into the geometry and nature of the emission processes. During SMM 1 in 1980, ultraviolet and X-ray observations were made with ~ 1 s time resolution; on SMM 2 they can be improved to within 0.1 s. This accuracy may permit SMM scientists to determine whether ultraviolet and X-ray emissions are a secondary result of a heating (thermal) process or a result of (nonthermal) electron bombardment of the chromosphere. The mechanism of generating the high-altitude coronal transience, which often follows flares, and its propagation in interplanetary space will also be studied, with the coronagraph polarimeter (CP). HXIS observations on SMM 1 indicated that the chromosphere brightens at X-ray wavelengths just as a coronal transient emerges but before the associated flare is observed. In some cases, transients follow a major flare, while in others they are associated with eruptive prominences. This raises the interesting question of whether the impulsive flare causes all the associated phenomena or whether it is a byproduct of a more fundamental process. A coordinated programme to observe these prominence eruptions, coronal transience, interplanetary and Earth effects will use several instruments on SMM 2 and other satellites and ground-based observatories.

The first accurate (to 0.005 per cent)

long-term observations of the solar constant, by the active cavity radiometer (ACRIM) on board SMM 1, showed that it declined by 0.04 per cent per year from February 1980 to July 1982. ACRIM has also shown that sunspots on the Sun's disc cause a decrease in the solar constant, although it is a puzzle where and in what form the 'missing' energy reappears. These two observations will be studied further and can be compared with the new series of solar constant measurements initiated on Space Lab 1 (*Science* **225**, 180; 1984).

GRS observations of the highest-energy solar flares show that a large fraction of them recur with a period of ~ 5.5 months (Rieger, E. *et al. Abstr. 25th Plenary Mtg of COSPAR*, 64; 1984). There is also evidence that the flare radiation pattern > 300 keV is anisotropic (Vestrand, W.P. *et al. Bull. Am. astr. Soc.* **16**, no. 2, 475; 1984). Longer-term observations on SMM 2 should reveal whether these features are characteristic only of the peak phase of activity before 1983 or whether they are a basic property of all energetic flares.

In early 1986, SMM 2 will temporarily interrupt its solar mission and all its pointed instruments will be focused on Halley's comet as it passes near the Sun in its perihelion approach. Never before has there been such an array of sophisticated instruments studying a comet, and the scientific rewards could be tremendous. Continued observations to the next maximum of solar activity (~ 7 years) may also be possible, and could produce further dramatic discoveries. □

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complements published accounts of Dorestad's topography, pottery, coins and jewellery. It confirms that this was no shanty town full of aspiring peasants, but a community which managed its agricultural resources with eminent good sense.

The traders, farmers and their families in Dorestad were far from the bread-line. Their diet was rich in protein, of which 30 per cent came from the consumption of animal foodstuffs. Prummel's statistical analyses of the archaeozoological data suggest that, on average, almost a third of the meat eaten in Dorestad was mutton and pork and 50–60 per cent was beef. Diet varied across the site, of course, with more pork being eaten in the village and more mutton being consumed by the merchants on the quayside.

Very little meat, it seems, was acquired by hunting or fishing. Instead, Prummel estimates an average household possessed about 10 cows (including 3 milk cows and 2 for slaughter), 10 sheep (2 producing milk and 2 for slaughter), 10 pigs (of which 3 were slaughtered each year), 20 chickens (5 laying hens, 6 for consumption) and a goose. A family of six to eight persons could have fared comparatively well on these animals alone. However, many of Dorestad's citizens were probably either part-time artisans or seasonal sailors, owning shares in the long-distance trading boats and their ventures, and so could have supplemented their income with certain luxuries⁵. Prummel suggests that some animal products might have been exchanged for fodder, though no study of the agrarian resources surrounding Dorestad is available to confirm this.

At Dorestad, as at most Early Medieval sites, the signs of butchery are relatively rare. The animals were probably slaughtered in the emporium and disjointed on the spot. Many long bones were split longitudinally, possibly for their marrow, and many bones bear cut marks. The absence of smaller bones implies that the meat was stewed in the coarse earthenware cooking-pots of the time. Indeed, archaeozoologists are coming to the conclusion that the Dark Age diet was dull, though rich in protein. In western Europe, it was only after the turn of the millennium that there was a return to the earlier Roman tradition of jointing meat, possibly to meet the demand for a more varied tastier cuisine⁶.

The animal bones from Dorestad and the comparable emporium at Southampton, and from Dark Age villages throughout Europe, challenge the primeval picture of Early Medieval life. Those at Dorestad generally betray the age profiles and sizes of well-managed stock. A significant portion of the cattle, sheep and pigs was kept until 3–4 years of age. The cattle were not the small thin creatures implied by some of the specimens found on north German and Viking-period sites at this time. Instead, they appear to have been stocky, owing something to their descent from the well-

Archaeology

Diet in the Dark Ages

from Richard Hodges

A DECADE ago the French historian Georges Duby, in a wide-ranging appraisal of Early Medieval Europe, asserted that "the feeble productive capacity" of those times "explains the abiding presence of famine, particularly in regions where men had adopted the custom of subsisting primarily on bread"¹. Duby's picture of a world of forests, primitive political relationships and impoverished agriculture was founded on a few royal and monastic sources. Much as he predicted, archaeology has offered a far more scientific base with which to examine Dark Age economics². Post-war excavations of trading emporia and villages in England, Italy, West Germany and the Netherlands offer new scope for reconstructing life in the Dark Ages. Indeed, the great wealth of data now assembled in Wietske Prummel's important monograph on the faunal

assemblage from the recently excavated Dutch emporium of Dorestad challenges all Duby's expectations³.

The largest settlement west of Constantinople in AD 800, the great emporium of Dorestad lay at the junction of the rivers Rhine and Lek, and acted as a port, serving the needs of the Carolingian aristocracy in the middle Rhineland in the eighth and ninth centuries. About a quarter of the settlement (forty hectares) has been excavated by the Dutch State Archaeological Service⁴. The rivers were lined with wooden jetties, behind which were warehouses, and in the centre of the site was a large village. More than 10,000 people must have lived here at its zenith when it was supplying Charlemagne with Anglo-Saxon and Scandinavian imports. Prummel's analysis of the large faunal assemblage retrieved in the excavations

managed herds of the Roman era⁷. The sheep and pigs, too, were comparatively large animals by Roman and Medieval standards.

The patterns indicate a regulated agricultural cycle. This is not altogether surprising as some Anglo-Saxon and Carolingian laws indicate that kings were as much farmers as warriors, and concerned about legislation on crop-rotation and stock-breeding as it affected tributes in kind made each year to them⁸. Interestingly, the age profiles of the stock are much the same in the emporia as they are in the countryside. Clearly, a mixture of common sense, central authority and low taxes helped to sustain good standards of husbandry at all levels of society. This contrasts with the last days of Roman control not only in Italy⁹, but all over

western Europe, by which time high taxes in kind had effectively destroyed stock management. □

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Control of translation

Enzymes and toxins that regulate protein synthesis

from Mike Clemens

DIPHTheria toxin and toxin A of *Pseudomonas aeruginosa* produce their effect in eukaryotic cells by inactivating elongation factor 2 (EF-2), an essential factor in protein synthesis. This protein of about 93,000 molecular weight catalyses the translocation of the growing polypeptide chain from the aminoacyl-tRNA site to the peptidyl-tRNA site on the ribosome each time an amino acid is added during chain elongation. The mRNA template is also shifted (by a distance of one codon), in a reaction that requires energy derived from the hydrolysis of guanosine triphosphate. Inactivation of EF-2 by the toxins is achieved by the transfer of a molecule of ADP-ribose from nicotinamide adenine dinucleotide (NAD⁺) to a modified amino acid on the factor, 2[3-carboxyamido-3-(trimethylammonio)propyl]histidine^{1,2} (Fig. 1), commonly known as diphthamide. Synthesis of this amino acid involves post-translational modification of a histidine residue in EF-2 by at least three enzymes^{3,4}. The assumption that diphthamide is not present in EF-2 solely for the purpose of allowing protein synthesis to be shut down during diphtheria infection is justified by recent results⁵.

Lee and Iglewski report that polyoma virus-transformed baby hamster kidney cells (pyBHK) contain an endogenous ADP-ribosyltransferase with a similar specificity for the diphthamide residue⁵. The enzyme from pyBHK cells appears to be functionally analogous to, but immunologically distinct from, fragment A of diphtheria toxin — the portion which has the mono(ADP-ribosyl)transferase activity. Similar enzymes may occur in a variety of other cell types since Lee and Iglewski

have also identified an EF-2-specific mono-(ADP-ribosyl)transferase in beef liver. It is unclear whether these enzymes have any relationship to the cellular enzymes or microbial toxins capable of ADP-ribosylating other protein substrates, such as adenylate cyclase⁶ or histones⁷, but the absence of diphthamide residues from such substrates suggests that the new ADP-ribosyltransferases represent a separate group.

Since ADP-ribosylation of EF-2 totally inactivates it (by inhibiting GTP-dependent translocation on the ribosome), it is possible that the enzyme identified by Lee and Iglewski has a function in the regulation of protein synthesis. Such a possibility is supported by the observation that the covalent modification of EF-2 by the enzyme is reversible, at least *in vitro* in the presence of fragment A and nicotinamide, and by the fact that pyBHK cells also contain an endogenous inhibitor of the EF-2-specific ADP-ribosyltransferase⁵. The presence of this inhibitor presumably ensures that a catastrophic inactivation of

all the EF-2 in the cell does not normally occur. Reversible covalent modifications of protein synthesis factors by, for example, phosphorylation are implicated in translational regulation in a number of cases⁸, although control of protein synthesis is normally exerted at the stage of polypeptide chain initiation rather than elongation.

The subcellular distribution and activity of EF-2 changes in cells in different physiological states. For example, there are differences between exponentially-growing and non-growing cells in culture⁹. Variations in EF-2 activity have been attributed to changes in the amount of the elongation factor protein. However, since quantification of EF-2 in those studies was based on the number of acceptor sites available for diphtheria toxin-catalysed ADP-ribosylation, another look at these phenomena may now be warranted. In the light of the new data, it is possible that ADP-ribose may already have been present on a fraction of the EF-2 as a result of the activity of the cellular enzyme, resulting in an underestimation of the total EF-2 levels.

Whatever the role of ADP-ribosylation of EF-2 in normal cellular physiology, it is clear that the diphtheria toxin fragment A represents a further example of a microbial product which exerts its effects by mimicking a normal cellular control process and subverting it to its own ends. Such a mechanism is consistent with the suggestion of Pappenheimer and Gill¹⁰ that the *tox* gene of the *Corynebacterium diphtheriae* bacteriophage β , which codes for the diphtheria toxin, was originally derived from a eukaryotic gene. □

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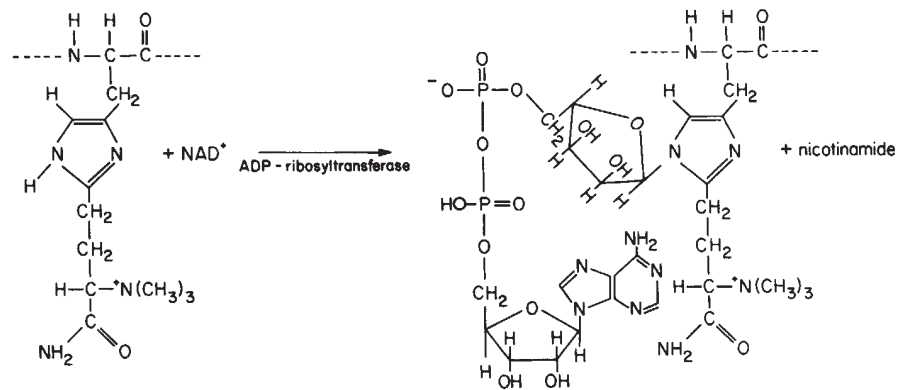


Fig. 1 Modification of diphthamide by ADP-ribosylation.

Genetics

Gene conversion in the absence of reciprocal recombination

from Gerald R. Fink and Thomas D. Petes

THE analysis of gene conversion between repeated eukaryotic genes has revealed some surprises about the basic rules of genetic recombination. Gene conversion is the non-reciprocal transfer of DNA sequences from one homologous gene to another. When gene conversion events are detected between allelic loci in fungal genetic systems, approximately 50 per cent of the events are associated with reciprocal recombination for flanking markers. So compelling is this 50 per cent rule that it is incorporated into virtually every current model of recombination. In higher eukaryotes, however, indirect evidence suggests that gene conversion events occur frequently but are not usually associated with reciprocal cross-overs. We discuss below two genetic studies^{1,2}, published in this week's *Nature*, which demonstrate that recombination between some classes of repeated yeast genes are conversion events that are not associated with reciprocal exchange. These and similar observations suggest that models of recombination in which gene conversion and reciprocal recombination are intimately related may require re-evaluation.

Gene conversion in fungi is typified by non-Mendelian meiotic segregation in which heterozygous alleles *A* and *a* distribute 3*A*:1*a* or 1*A*:3*a* instead of 2*A*:2*a*. The simplest explanation for conversion at the molecular level is that a DNA segment from one chromosome is transferred to the homologous chromosome, replacing the resident segment^{3,4}. This transfer of information occurs without loss of the segment used as a template.

Most of the evidence for gene conversion in higher eukaryotes has been obtained by DNA sequence analysis of small families of repeated genes whose sequences have diverged (such as the genes encoding the haemoglobins, immunoglobulins and proteins of major histocompatibility loci)⁵⁻⁸. For example, Weiss *et al.*⁸ found that a mutant allele of the mouse *H-2K^b* gene (known as *H-2K^{bml}*) contained a clustered series of changes in the middle of the gene; the same bases were present in the comparable position in the non-allelic *H-2L^d* gene. Since the sequences flanking the *H-2K^{bml}* gene were unchanged, Weiss *et al.* suggested that the variant arose as a result of a non-reciprocal recombination event in which the *H-2L^d* gene may have acted as the donor template. It has also been suggested⁹ that a high frequency of gene conversion between repeated genes within a family of related sequences could account for the sequence conservation observed in many repeated gene families.

Many geneticists are troubled by the analogy between recombination in higher eukaryotes and gene conversion in fungi because conversion in higher eukaryotes is apparently unassociated with reciprocal recombination, whereas fungal gene conversion events are associated with reciprocal recombination of flanking markers approximately 50 per cent of the time¹⁰. If gene conversion events between repeated genes of higher cells were associated with reciprocal recombination, one might expect that half the time they would give rise to chromosomal deletions, inversions or translocations. The absence of these chromosome aberrations in association with the non-reciprocal events documented in higher cells leads one to think that gene conversion in fungi is a fundamentally different process.

Two papers in this issue describe gene conversions in yeast that are not associated with reciprocal recombination. Previous studies¹⁰ that established the 50 per cent rule dealt only with recombination between alleles; the new work^{1,2} concerns recombination between duplications on the same chromosome.

Klein¹ reports on meiotic gene conversion between inverted duplicated *HIS3* genes. She finds that intrachromosomal gene conversions between the duplicated genes are not associated with reciprocal exchange. These data are in agreement with recent studies that show a lack of association between intrachromosomal conversion of direct repeats and reciprocal exchange^{11,12}.

In a second paper, Klar and Strathern² describe mating type interconversions that occur in mitosis. The mating type switch in yeast involves a gene conversion-like event in which genetic information at either the *HML* or the *HMR* locus is substituted for the allele residing at *MAT* without the loss of the donor locus. Since *HML*, *HMR* and

MAT are repeated DNA segments on the same chromosome, the mating type interconversions are intrachromosomal gene conversion events like the meiotic conversions studied by Klein. Klar and Strathern report the striking result that although intrachromosomal mating type conversion is not associated with reciprocal recombination, conversion events between two *MAT* loci at allelic positions on homologous chromosomes show a significant association with reciprocal recombination of flanking markers.

These results raise the possibility that there is a single unifying picture of gene conversion applicable to fungi and higher eukaryotes. The simplest view is that only recombination events involving two homologous chromosomes are constrained by the 50 per cent rule, whereas intrachromosomal events (whether intrachromatid or sister-chromatid exchanges) are free of reciprocal recombination both in mitosis and meiosis. Indeed, the gene conversion events observed in higher cells often appear to occur within multi-gene families located on the same chromosome and qualify as intrachromosomal events.

Two of the experiments reported by Klar and Strathern do not fit exactly into this unifying picture. They show that *HMR* to *MAT* conversions are not associated with reciprocal recombination even when the two loci are on different chromosomes. This result argues against a simple dichotomy between intrachromosomal exchange and exchange involving two homologues. Klar and Strathern argue that it is the extent of sequence homology that determines whether a conversion event will be resolved as a reciprocal recombination event; Carpenter¹³ has made a similar suggestion to explain *Drosophila* recombination data. By this view, in both intrachromosomal conversion events and conversion events involving homologous chromosomes, when there is little flanking homology, there will be little reciprocal exchange. Yet Klar and Strathern find conversion events associated with unequal sister-strand reciprocal recombination even where there is no extensive flanking homology. Moreover, other yeast studies¹⁴⁻¹⁶ have demonstrated mitotic conversion between repeated genes on non-homologous chromosomes associated with reciprocal recombination. It is possible that some of these disparate results were influenced by the unusual genetic constructions required to study the conversion event or the sequence of the interacting alleles.

Despite these apparent contradictions, there is now clear documentation of gene conversion unassociated with reciprocal recombination in both mitotic and meiotic cells of yeast. Removal of the obligatory association between conversion and crossing-over should have a salutary effect on the whole field of recombination. We are now free to consider the possibility that the 50 per cent rule does not imply a

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mechanistic association between conversion and recombination. Perhaps these are two fundamentally different events. The 50 per cent association could be merely that — an association between two different events caused by the highly recombinogenic nature of the substrate. Whether it is the topology of the interaction, the extent of homology, the sequence of the interacting genes or a combination of these factors that determines whether a reciprocal recombination will occur remains a question for future research. □

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100 years ago

WATER BELLS

THE accompanying design (from *La Nature*) represents a water bell, the invention of M. Bourdon, of more transparent and complete effect than those hitherto produced.

The bell to which we here call attention is distinguished by the way in which it causes the liquid vein to expand as soon as it reaches the orifice of the ajutage. Instead of making it strike against a metal plate, the surface of which, however carefully polished, always betrays some imperfection, M.E. Bourdon brings an antagonistic column of water to bear on it, so that the jet expands and falls into the basin, forming a bell as clear as crystal and impervious enough to cut off all communication between the interior and circumbient air.

The pipe conducting the water from the reservoir ends in a truncated form of nozzle, of about 12 degrees of angular opening, in such a way as to make all the threads of water converge towards the middle of the jet. Over this is placed

Mathematics

Three conjectures in one blow

from Ian Stewart

ALTHOUGH it may not always be apparent to the casual observer, a great deal of modern mathematics is inspired by the time-honoured problem of solving equations, particularly those defined by polynomials. Early work on curves and surfaces defined in coordinate form by such equations led to the flourishing field of algebraic geometry; the problem of solving the equations using only whole numbers led to Diophantine analysis and algebraic number theory. It might be expected that the subject would by now

concentrically to the truncated nozzle a glass tube of about 20 cm. in length, and of the same interior diameter as that of the orifice by which the water rises from the reservoir.

The apparatus so arranged and the basin filled with water to the level of the overflow, the cock will be gently opened, and the water traversing the interval between the ajutage and the tube will rise a few centimetres high in the latter. A ball of ovoidal shape will then come to view. By opening the cock very slowly its diameter will gradually enlarge till the bell assumes the form of a hemisphere. At this point let the opening of the cock be reduced a very little, and the bell will change its shape; its rim will become lowered to the plane of the water of the basin, and its profile will show a bell similar to the gardener's bell glass.

By placing a very thin copper wire vertically towards the top of the bell, a vertical incision may be made in the bell, parting it into two separate sides. Through the gap thus formed, a statuette, a lighted candle, or a cage containing a bird, may be introduced inside the bell without wetting it. The tubes hitherto used have not exceeded 20 mm. in diameter; but by employing apparatus of much larger dimensions, water bells of from 3 to 4 metres in diameter might be produced just as perfect as those of from 60 to 80 centimetres, and under which people might walk about or lounge at pleasure.

From *Nature* 30, 408, 28 August 1884.

have been studied to death, but the reverse appears to be true: after some five centuries of work we are only beginning to glimpse the wonders concealed in its hitherto murky depths.

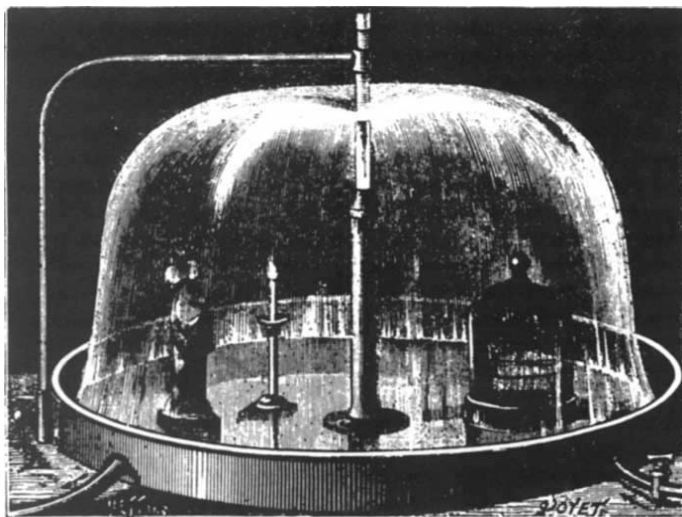
In any mature branch of mathematics that is not played out, there will be a number of plausible guesses — usually dignified as conjectures — about the way the subject should fit together. The proof of a notorious conjecture is one of the most celebrated mathematical events. It establishes the merits of new methods and the abilities of new mathematicians; it dispels confusion and makes previously intractable problems look easy. It is understandable, therefore, that the mathematical world should be unusually excited by the vanquishing of not one, but three important conjectures at a single stroke (*Invent. Math.* 73, 349; 1983). The dragon-slayer in question is the young German mathematician Gerd Faltings; the conjectures bear the names of Mordell, Tate and Schafarevich. Almost in passing, the results deal a severe (but not quite mortal) blow to the most notorious unsolved problem: Fermat's Last Theorem.

Pierre de Fermat stated (without proof in a marginal note) that the equation $x^n + y^n = z^n$ has no nonzero solutions in whole numbers x, y, z , whenever $n \geq 3$. Made in the mid-seventeenth century, this statement remains unproved — although it is known to be true for the first few hundred thousand values of n . A simple corollary of Falting's new work is that for $n \geq 3$, there are at most a finite number of solutions to Fermat's equation. This is the first time anyone has proved a significant fact about Fermat's equation that is true for all values of n , and it raises hopes that a complete solution may be found.

To see the connection with algebraic geometry, observe that the Fermat equation can be written as $X^n + Y^n = 1$, where $X = x/z$ and $Y = y/z$. If x, y, z are whole numbers, then X and Y are rational — and vice versa. Hence Fermat's Last Theorem is equivalent to the statement that the plane curve $X^n + Y^n = 1$ contains no points (X, Y) with rational coordinates. Thus, it is a problem about the arithmetic of curves.

Associated to any curve is a topological invariant called its genus. Roughly, by allowing the variables to become complex numbers, the set of solutions can be extended to form a surface embedded in a two-dimensional complex space. Topologically, any such surface is just a multi-holed doughnut, and the genus is the number of holes. For the Fermat equation, the genus is $\frac{1}{2}(n-1)(n-2)$.

In 1922 L.J. Mordell conjectured that



any irreducible (that is, not factorizable) polynomial equation in two variables, of genus 2 or more, could have only a finite number of rational solutions (*Proc. Cambridge phil. Soc.* 21, 179; 1922). He did so on the most slender evidence, and for a long time the conjecture was considered to be just one of those things that would make the world nicer if it were true — but could just as easily turn out to be false. For example, the finiteness result for Fermat's Last Theorem follows immediately, since the genus $\frac{1}{2}(n-1)(n-2)$ is at least 3 if $n \geq 4$, and the case $n = 3$ was proved by Euler in about 1738. In 1968, however, the Russian mathematician A.N. Parshin (*Math. USSR Izvestija* 2, 1145; 1968) showed that Mordell's conjecture would follow from another conjecture due to I.R. Shafarevich, about the behaviour of equations under 'reduction modulo a prime', and that conjecture was more plausible.

Faltings has proved the Shafarevich conjecture — and hence also that of Mordell — by a massive mobilization of the available machinery of algebraic geometry augmented by some crucial new ideas of his own. Treading an established path, he

generalizes the problem from curves to higher-dimensional objects known as abelian varieties. He introduces a measure of the complexity of such a variety, named its height, and finds a formula describing how the height changes if the variety is replaced by a different but similar one (resulting from a technical operation known as an isogeny). One implication of this formula is a conjecture due to J. Tate (*Invent. Math.* 2, 134; 1966) about mappings between abelian varieties.

The road to a finiteness result is now open: first, show that only a finite number of the relevant objects have a given height; and second, show that the heights themselves are bounded by some definite upper limit. For the Shafarevich conjecture the relevant objects turn out to be 'principally polarized abelian varieties of a given dimension having good reduction except for a given finite set of primes'. The finiteness is established using yet another series of conjectures, made by André Weil, and proved by Pierre Deligne in the late 1970s. □

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Neurobiology

Cross-section of the spinal cord

from Jennifer Altman

The London Spinal Cord Club is unusual as its membership is based on an interest in the tissue investigated rather than the academic disciplines of the investigators. Its recent symposium* reflected this in the wide range of approaches and techniques, from cDNA probes and voltage-clamp recording to studies on clinical patients.

Two very different conceptual approaches were in evidence: on the one hand a reduction of the system to its minimum, using tissue culture, tissue slice or isolated cord preparations for intracellular recording, and dealing with the physiology and pharmacology of single units; on the other hand, a move back towards studying populations of neurones *in situ*. The first approach is necessary for studying the membrane effects of putative transmitters (M. Mayer and G. Westbrook, NIH, Bethesda) and the interactions between drugs, transmitters and receptors (A. King *et al.*, St Bartholomew's Hospital, London). Isolated cord preparations have also been used for examining changes in electrical properties of rat sensory neurones as they mature (B. Fulton, University College, London) and for identifying abnormal neurone functions in the *spastic* mouse mutant (T. Biscoe and M. Ducher, University College, London). Such studies do little, however, to advance our understanding of how the spinal cord

works as a system, integrating inputs to produce defined and precise motor outputs. It is here that the whole-animal investigations are essential, and modern neuroanatomical and neurochemical methods of tracing and mapping neuronal populations are proving powerful tools for this type of analysis.

Numerous populations of neurones can now be identified because they contain specific marker molecules, including enzymes and peptides. The development of cDNA probes for tissue-specific sequences (J. Dickson *et al.*, Institute of Neurology, London), now in its infancy, promises to be an important tool for characterizing neurones. But the question of the functional significance of the markers remains open. A link between histochemistry and function has been found for glycogen phosphorylase (C. Woolf *et al.*, University College, London), the active form of which is found only in stimulated neurones and therefore identifies active cells. However, in the case of peptides identified in primary afferents, there is still no good evidence that they have specific transmitter or modulator effects on postsynaptic cells, as has been shown for cholecystokinin in cortex (J. Kelly, St George's Hospital, London). The demonstration that developing motor neurone terminals contain β -endorphin, an opioid peptide, that disappears as the neurones mature but reappears when their axons are damaged (L. Haynes *et al.*, Birmingham Medical

School and National Institute of Medical Research, London), raises the possibility that some peptides could be left over from development or be involved in synaptic maintenance. Haynes *et al.* went on to show that β -endorphin has a neurotrophic effect on cholinergic transmission by regulating the type of acetylcholinesterase in the synaptic cleft.

The great advantage of the spinal cord over other parts of the central nervous system for studying neuronal integration is that the outputs can be monitored at the same time as inputs are manipulated. Between the inputs and the outputs, however, lie complex networks of interneurons, which are still relatively inaccessible to investigation. Nonetheless, E. Jankowska (Göteborg) has identified interneurons of particular reflex pathways using transneuronal transport of horseradish peroxidase conjugated with wheat germ agglutinin. This work, together with results from S. McMahon (University College, London) on the distributions of C fibre terminals in skin and muscle and from S. Hunt (MRC Neuropharmacology, Cambridge) showing that cutaneous afferents containing substance P terminate more dorsally than those marked by fluoride-resistant acid phosphatase, begins to establish a picture of functional subdivisions within the laminae of the cord.

Multiunit recording from neurone populations requires sophisticated statistical analysis and interpretation is very difficult. How significant is a small relatively rare event such as the monosynaptic input from bulbo-spinal interneurons to intercostal motor neurones (P. Kirkwood *et al.*, Institute of Neurology, London)? Recordings from single identified motoneurons in the locust during the generation of rhythmic behaviour such as flight show that inputs of only 1–2 mV can advance the neurone's firing providing they occur at the right time in the depolarization cycle (Robertson, R.M. & Pearson, K.G. *J. comp. Neurol.* 215, 33; 1983). This observation supports Kirkwood's suggestion that spinal motoneurone outputs may be subtly influenced by quite small changes in the timing of the inputs.

Lastly, the presence of two paraplegic observers from the International Spinal Research Trust meant that the pathological applications of work on the spinal cord were kept in mind. Research into reflex pathways in humans, both normal subjects and patients with clinical disabilities (B. Day *et al.*, Institute of Psychiatry, London; J. Iles and R. Roberts, Universities of Oxford and London; T. Davies, Guy's Hospital, London) is confirming results from animal studies and should lead to improved diagnosis, although the meeting had little immediate to offer towards the prevention or cure of spinal cord malfunction. □

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*Recent advances in the spinal cord: a Brain Research Association symposium, held at the Centre for Neuroscience, University College London, 6–7 July 1984.

How did retinoblastoma arise?

SIR — We read with interest the article by Kyritsis *et al.*¹, in which they suggest that retinoblastoma may have arisen from a primitive neuroectodermal cell. We have studied the properties of the glycolytic enzymes pyruvate kinase, hexokinase, aldolase and enolase in these tumours, and it is interesting to compare our data with the work of Kyritsis *et al.*

In normal fetal retina, five different forms of pyruvate kinase could be detected by electrophoresis (K_4 , K_3M , K_2M_2 , KM_3 and M_4)². In retinoblastoma, the M_4 isozyme is completely absent, whereas in normal adult retina the K_4 isozyme is almost completely absent. In normal retina, both fetal and adult, hexokinase type I predominated; retinoblastoma was characterized by the presence of hexokinase type II. The study of aldolase in retinoblastoma revealed a large predominance of the fetal type (for example A-type) in contrast with normal retina. Subsequent studies³ investigating the same enzymes in medulloblastoma and neuroblastoma gave support to the hypothesis that these tumours have a common embryonic origin with retinoblastoma. In a study of enolase⁴ we found that neuroblastoma, medulloblastoma and retinoblastoma are characterized by the presence of all three types of enolase, namely $\alpha\alpha$, $\alpha\gamma$ and $\gamma\gamma$.

Thus we think that the results of Kyritsis *et al.*¹ and our findings lead to the same conclusions.

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Sex and generations of primroses

SIR — The highly evolved heterostyle incompatibility system of primroses which, in most populations, ensures almost complete outcrossing, must surely be of sufficient importance to the primrose for it to have protected itself from an easy regress to an inbreeding species due to the rapid increase of homostyles, readily produced by mutation or, more probably, recombination. The controversy concerning the rate of self-fertilization of homostyle

primroses, in which I was involved a generation ago and which is rekindled in the pages of your journal by Piper *et al.* (*Nature* **310**, 50; 1984) and the News and Views item by Richards in the same issue (*Nature* **310**, 12; 1984) is thus of some considerable importance for the sex life of the primrose. Richards castigates both myself and Crosby for a lack of good data while, at the same time, quoting both of our extensive population studies and my own careful investigations into homostyle protogyny in my first ever published paper (*Heredity* **12**, 363; 1958). These and later studies clearly showed that the homostyle stigma was receptive to pollen well before anthers dehiscence, and provided a likely explanation for a much greater frequency of outcrossing by homostyles than might otherwise be expected. Thus the observation of large amounts of homostyle pollen on homostyle stigmas is not, by any means, conclusive evidence for self-fertilization. The earlier arriving thrum pollen may successfully compete with the later arriving homostyle pollen, as Richards indeed acknowledges.

I, too, have collected extensive data on seed counts per capsule and, using dominantly inherited flower colour markers had begun to obtain, in collaboration with Vivian Fyfe, direct estimates of the frequency of homostyle outcrossing. But I was diverted from these interests to other equally important areas of research. Indeed I had hoped that in the quieter years following my eventual retirement, perhaps a primrose generation or two in the future, these observations could have been extended and eventually published together with observations on the frequencies of homostyles in an ordinary population of primroses on the banks of Clare College, Cambridge, to which I had added a few homostyles in the late 1950s. But now the present excitement has preempted these aspirations.

It was already clear from my own observations that the frequency of outcrossing might vary considerably according to the ecological conditions and, of course, also from year to year especially as a function of climate. Thus, observations in any one place or at any one time are clearly subject to considerable variation. In my extended discussion of this issue, including a comprehensive theoretical treatment (*Phil. Trans. R. Soc. B* **242**, 517-549; 1960), I pointed out, following Mather and others, that the gradual evolution of an incompatibility system would be likely to have led to some form of partial self-incompatibility before the final evolution of the incompatibility switch mechanism itself had been perfected. This would account, for example, for the protogynous phase observed in the homostyles, that itself could account for the tendency of the homostyles to cross-fertilize more than expected. The crucial question is, what limits the increase in frequency of homostyles in most populations in which they

arise? (In collaboration with E.B. Ford, it was clearly shown that predominantly pinthrum populations did contain rare homostyles.) Crosby assumed that, given their enormous advantage due to self-fertilization, especially if this was complete or almost so, it must be viability disadvantage that limits their increase. The theory, however, demands extraordinarily low viabilities which appear to be inconsistent with the observed ratios from breeding experiments. On the other hand, it can be shown that a reasonable degree of cross-fertilization by the homostyles can prevent their increase, given only a moderate viability disadvantage. This result, to my mind, was compatible with the overall data and provided a simple explanation for the protection of the heterostyle outcrossing system against invasion by variant inbreeding homostyles.

The perturbation theory used to analyse the complex population genetic models underlying this system clearly foreshadowed subsequent analyses of 'protected' equilibria and 'evolutionarily stable strategies' by John Maynard Smith and others. Furthermore the paper in *Phil. Trans.*, together with an accompanying paper by Crosby, were heralded in the pages of your journal as being amongst the first applications of computers to genetics and, more specifically, to the analysis of complex population genetic models. So, whatever the rights and wrongs of the theory and data, the problem stimulated new approaches to the analysis of complex evolutionary systems. Computer applications have clearly advanced more quickly in the last quarter of a century than work on the sex life of the primrose. Hopefully, another generation will be enough to give rise to a convincing and conclusive answer to the important question, for the primrose, as to what limits the increase in frequency of homostyles.

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A weighty problem cracked

SIR — I am writing to tell you about a discovery which seems far-reaching in its implications, and could well throw light on a number of outstanding problems in physics, such as why dropped toast always falls marmalade-down.

I must make it clear that I am not a physicist in the generally accepted sense of the word. To be honest, I am not a physicist in any sense of the word. Anyway, here we go.

It all started when I had a slight accident. I fell over in the saloon bar at the Groveling Toad and struck the back of my head on the space invaders machine. After two or three more pints I was back to normal, except for a severe pain in the neck which I could only relieve by crouching down and

holding my head on one side. This made life rather difficult, including watching television. It was this particular problem which led one evening to my laying the set on its side so that I could see it more clearly from my awkward position.

After an hour's viewing in this way I noticed that the colours on the screen appeared to be running. One side of the figures on the screen had a red shadow, and the other side a blue shadow, or, of course, since the set was on its side, the blue shadow appeared on the upper edges and the red shadow on the lower. Returning the set to the upright position cured the problem.

I thought about this for a bit, and I was about to settle for the probability that some bits had come loose inside the set when it occurred to me to carry out a little experiment. I laid the television set on its other side. I left it for half an hour and then returned, confidently expecting that I would now see the red shadow at the top and the blue at the bottom — but no! The red was at the bottom again!

This called for a lot more thought. Hypothesis after hypothesis raced through my mind, each to be rejected as fast as it occurred on the grounds of inconsistency, impossibility, or downright stupidity. Eventually only one hypothesis remained, and this was:

Colours have weight, and red is naturally heavier than blue.

Thus the television set, not being adjusted for sideways operation, did not compensate for the weight of the colours.

I realized at once the possible implications, but, first of all, more evidence was required. To rush out and stop people in the street to tell them that red is heavier than blue, particularly when one is obliged to adopt a somewhat hunched-up stance with one's head on one side, might have invited comment of the worst sort, if not actual arrest. No, proof had to come first. I had to devise some experiments to support my hypothesis.

I decided to use as an experimental model a tomato plant, of which I had several at the time, growing against a sunny wall. I selected a medium-sized unripe tomato, hanging from a strong branch, and checked it for uniformity of greenness (I assumed that green would do as well as blue). I then measured its mean diameter, and the height above the ground in which it grew, and then just waited. It took a long time to ripen, but eventually it became uniformly red. I eagerly measured its height above the ground once more and discovered it to be a quarter of an inch lower than when it was green! The red tomato was heavier than the green!

But was this valid? I hastened to re-measure the size of the tomato in order to see if it had sneaked on a bit more mass in the intervening days, but it was here that things went wrong. The tomato had become much softer by this time, and my engineer's calipers were too much for it. It

collapsed in a squashy heap on the ground.

What I needed was a bit more sensitivity, so I devised a much more sophisticated experiment involving a vice, a razor blade, a 1-foot ruler (white), a saucepan, two desk lamps, and some sheets of red and blue plastic film. I fixed the razor blade in the vice, and balanced the ruler across it. (Have you any idea how difficult it is to balance a plastic ruler on a razor blade?) I then stuck some red plastic film over each of the desk lamps and carefully positioned them so that one shone on each end of the ruler. I forget what the saucepan was for. I then switched on the lamps and both ends of the ruler glowed red as it hung there on the razor's edge. I waited about a minute, during which the ruler remained balanced, and then I suddenly replaced one of the red plastic sheets with a blue one. For about twenty seconds the ruler remained where it was, one end red, the other blue. Then slowly the red end began to sink, and the ruler fell off!

Of course, I could not let the problem drop there. All sorts of questions clamoured to be answered, for instance, what is the order of weight of the colours? I set out to determine this by repeating the balancing experiment a number of times with differently coloured plastics, and after many hours of delicate balancing I managed to discover the relative weights of the colours. In order of weight, lightest first, they are: white, yellow, green, blue, orange, pink, red, black.

I have other experiments under way now, some involving coloured balloons filled with hydrogen sulphide, others involving the production of coloured bubbles from a mixture of detergent and Dulux emulsion, and, although the neighbours are beginning to complain, the results are looking very promising.

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The hand of *Archaeopteryx*

SIR — Hecht and Tarsitano¹ defend their interpretations of the homologies and morphology of the hand of the protobird *Archaeopteryx* against what they believe are distorting and misrepresentative criticisms by Howgate². However, Howgate is essentially correct on the issues. It is Hecht and Tarsitano who are misreading the data.

The hands of *Archaeopteryx* appear to be very similar to those of their potential ancestors, the predatory theropod dinosaurs. Hecht and Tarsitano suggest this resemblance is only superficial because "the digits of birds must be 2-3-4 based on embryological work"^{1,3-5}, while palaeontologists identify the digits in derived theropods as 1-2-3. Hecht and Tarsitano then ask "do the similarities observed by palaeontologists have greater weight than the evidence derived from

ontogeny by embryologists?"¹. What they fail to note is that Hinchliffe, whom they repeatedly cite in support of their argument, has cautioned that, "the embryological convention that digit 1 is missing (in bird embryos) is not based on firm evidence"⁶. Even bird embryos never have more than four digits, so there is no way to establish which digits really are present. Such unreliable data cannot be used to disprove the homology of theropod and bird hands, hence the palaeontological evidence does outweigh the embryological work. If theropods are bird ancestors, then the progressive loss of the outer digits from early dinosaurs to derived theropods shows that *Archaeopteryx* and birds retain digits 1-2-3.

Hecht and Tarsitano also continue to defend the possibility that the disarticulated joint between phalanges 1 and 2 on manus digit III is actually a break^{1,3-5}. The problem is that there is no evidence to support this idea. Having examined the Berlin specimen, I can confirm that the particular surfaces at this join are well preserved. Besides, this joint is present in all five of the articulated hands preserved in three specimens. Howgate was caustically critical of Tarsitano and Hecht's speculations on how these hands came to be "broken"². This is understandable, for the idea that all these hands suffered identical injuries is simply beyond reason.

The criticisms that Hecht and Tarsitano direct at the theropod-bird hypothesis are based more on procedural grounds than on the data. This is annoying since Hecht and Tarsitano are not themselves innocent of such methodological errors. It is also putting the cart before the horse. How the theropod-bird hypothesis has been erected and defended is not nearly as important as its validity. As for the hand of *Archaeopteryx*, palaeontological evidence shows it to be theropodian in design and homology.

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Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a rather technical character which are not provoked by articles or letters previously published (where Matters Arising remains appropriate).

Cooling flows in clusters of galaxies

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Cooling gas in the centre of many clusters of galaxies is compressed and pushed inward by the thermal pressure of the hot, uncooled outer gas. Gravity acts as a focusing agent until the gas has cooled to galactic temperatures. The cooling process is thermally unstable, giving rise to optical filamentation. Once the gas cools below 10^4 K it presumably forms stars which add to the mass of a central galaxy. Accretion rates of up to $400 M_{\odot} \text{ yr}^{-1}$ are inferred from X-ray measurements so that the mass of such central galaxies may be due to cooling flows. Their optical and UV appearance emphasize that the gas predominantly turns into low-mass stars with an initial mass function unlike that of the solar neighbourhood. Optical and X-ray observations of cooling flows can be used to investigate the continuing formation of the largest known galaxies.

MANY clusters of galaxies are strong X-ray sources with luminosities of 10^{42} – $10^{45} \text{ erg s}^{-1}$ (ref. 1). Detailed spectra^{2–4} show that the emission originates primarily as thermal bremsstrahlung from a diffuse, intracluster gas with densities of 10^{-2} – 10^{-4} cm^{-3} and temperatures of 10^7 – 10^8 K. The mass of the gas is comparable to the mass of all the stars in all the galaxies of a cluster, but stars and gas together account for only 10–20% of the total cluster mass, most of which is, as yet, unseen and unidentified.

The hot gas sits in near hydrostatic equilibrium in the gravitational potential of the cluster, so that its density peaks in the cluster core, which is typically 150–250 kpc in radius. In this core, the radiative energy loss due to the emission of the observed X rays can have a significant effect on the behaviour of the gas. The loss may be sufficient to cool the gas and initiate a slow, subsonic, inward flow—the ‘cooling flow’ that is the subject of this review.

In a cluster, the onset and properties of the flow are determined by the properties of the cluster as a whole. Before it cools, the speed of sound in the gas, c_s , is comparable with the line-of-sight velocity dispersion of the galaxies in the cluster σ_c . Individual galaxies generally have internal line-of-sight velocity dispersions $\sigma_g \ll \sigma_c$, which means that the effect of their gravity on the intracluster gas is initially negligible⁵. Gravitational accretion⁶ onto a central galaxy can occur only after the gas has cooled considerably. Most clusters with cooling flows do contain central dominant galaxies that are receiving the infalling material, which passes through a brief stage of visibility when optically luminous filaments are produced by thermal instabilities^{7–9}.

The relevance of radiative cooling in the interpretation of X-ray observations of intracluster gas has been realized independently by various workers^{7,8,10}, although earlier work^{5,11} had noted that the cooling time of the intracluster gas in many clusters was similar to the age of the Universe, and Silk¹² had proposed the creation of a massive cluster galaxy due to cooling of primordial cluster gas.

It is only within the core of a cluster that cooling is observed to be important (the cooling time is less than the age of the Universe). The resultant flow is a negligible drain on the vast reservoir of hot gas beyond that, but it exceeds the small rate of stellar mass loss by the galaxies in the cluster core. There is no steady-state flow, such as the ‘radiative regulation’ proposed

by Cowie and Binney¹⁰, which would require cooling and mass injection to be important and to balance throughout the cluster. Nevertheless, the results they obtained do give a good description of the gas behaviour if applied only to the region where cooling is important. This cooling region was calculated by two of us (A.C.F. and P.E.J.N.⁷) to explain the high X-ray surface brightness of the Perseus cluster core revealed by moderate resolution pointed observations made with the Copernicus satellite¹³. The temperature and density profiles beyond the cooling flow region are then treated as initial conditions related to the formation and evolution of a cluster.

Individual galaxies not in cluster cores may have radiative inflows from interstellar gas^{14,15}. There is, as yet, no evidence for accretion from a general intergalactic medium^{16,17}. Heating and cooling of interstellar gas may together drive some form of gas flow in our Galaxy (the galactic fountain^{18,95}). Cooled gas accreted to the nucleus of a galaxy could power a central engine^{15,19,20}.

Evidence for cooling flows

There are now at least 27 clusters for which empirical evidence of cooling flows exists (Table 1). The nature of the evidence and appropriate references are discussed below.

A primary indicator of a cooling flow in a cluster is a highly peaked surface brightness profile (see Fig. 1). Such a distribution can imply that the time required for the gas in the central region to cool by radiating all its thermal energy is short compared with a measure of the lifetime of the system. The latter is generally taken to be some modest fraction of the Hubble time $t_H = H_0^{-1}$, where H_0 is the Hubble constant (which we take to be $50 \text{ km s}^{-1} \text{ Mpc}^{-1}$, so $t_H = 2 \times 10^{10} \text{ yr}$; because H_0 enters into the determination of t_c , t_c/t_H scales as $H_0^{1/2}$).

The connection between the X-ray surface brightness profile and the cooling time (t_c) is relatively model independent. The observed count rate per unit area in imaging detectors (for example, the Einstein satellite Imaging Proportional Counter (IPC)—see ref. 21) is sensitive primarily to the emission integral $\int n^2 dl$ along the line-of-sight (n is the gas density and l is the path length). It is relatively insensitive to the temperature of the plasma over the range 1–6 keV, just the range that is relevant for intracluster gas^{1,4}. In detail, the cooling time t_c for gas at temperature T that appears in an X-ray image as a region of

excess surface brightness $\Delta\Sigma$ with dimension l is then given by

$$t_c \equiv \frac{3kT\epsilon^{1/2}(T)}{(\Delta\Sigma/l)^{1/2}\Lambda(T)} \quad (1)$$

Here $\epsilon(T)$ is the instrumental sensitivity ($\epsilon = \int n^2 dl / \Delta\Sigma$ for a given T), $\Lambda(T)$ is the total emissivity of a plasma at temperature T (see ref. 22), k is Boltzmann's constant and we take the dimension l as an estimate of the depth of the region. This expression is plotted in Fig. 2 for various temperatures and for illustration we show a point computed using data²³ for the poor cluster MKW3s. The deduced value for t_c agrees well with the 8×10^9 yr found using a more sophisticated deprojection technique to determine the gas density. In general, the estimate of t_c derived in this manner will be an upper limit to the true value because the finite angular resolution and signal-to-noise ratio of most images under represents the true central surface brightness. This is indeed the case for MKW3s, for which an analysis²⁴ of High Resolution Imager (HRI) data gives t_c four times smaller than that deduced from the IPC image.

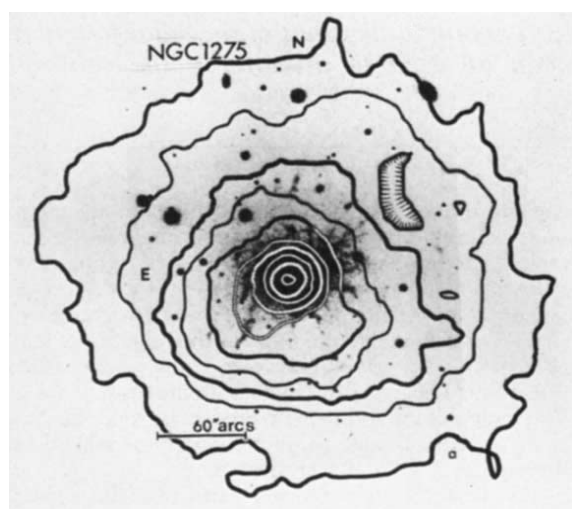


Fig. 1 X-ray emission contours of NGC1275 from the Einstein Observatory HRI image²² superimposed on the $H\alpha$ photograph of Lynds⁷⁹. An unresolved (<4 arc s) source is coincident with the nucleus.

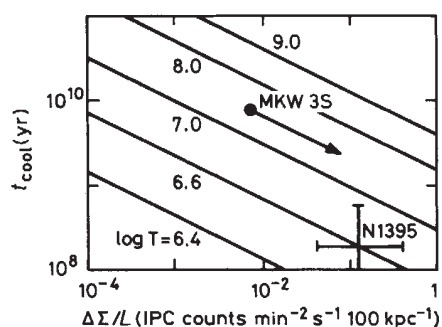


Fig. 2 Relationship between gas cooling time and excess surface brightness measured in units appropriate to the Einstein-IPC. The result²³ for the poor cluster MKW3s is shown. A shorter cooling time is derived²⁴ from the HRI due to the increasing surface brightness at smaller radii (arrow). Values¹⁵ for the isolated elliptical galaxy NGC1395 are indicated.

The high density implied by a high central X-ray surface brightness may also require that the temperature of the central gas be lower than that of the outer plasma. Otherwise, an absurdly high binding mass must be invoked to maintain the high-pressure gas in quasi-hydrostatic equilibrium (see ref. 24). Such a temperature inversion further reduces t_c and is itself indicative of a cooling flow²⁵. A rough estimate of the mass inflow rate, $\dot{M}$, is obtained by dividing the mass of cooling gas by its cooling time.

The high X-ray surface brightness around the giant elliptical galaxy M87 in the Virgo cluster suggested to Gorenstein *et al.*²⁶ and Mathews²⁷ that accretion was taking place. Since then, the greatly improved images obtained with the Einstein IPC have verified this conclusion and showed that 27 clusters, both those rich and poor in galaxies, have the very short central cooling times indicative of cooling flows (see Table 1). For many of these, the radial surface brightness profile can be analysed in more detail following a deprojection method developed by Fabian *et al.*²⁸ (Fig. 3). The derived density profile is very insensitive to the assumed parameters over their plausible

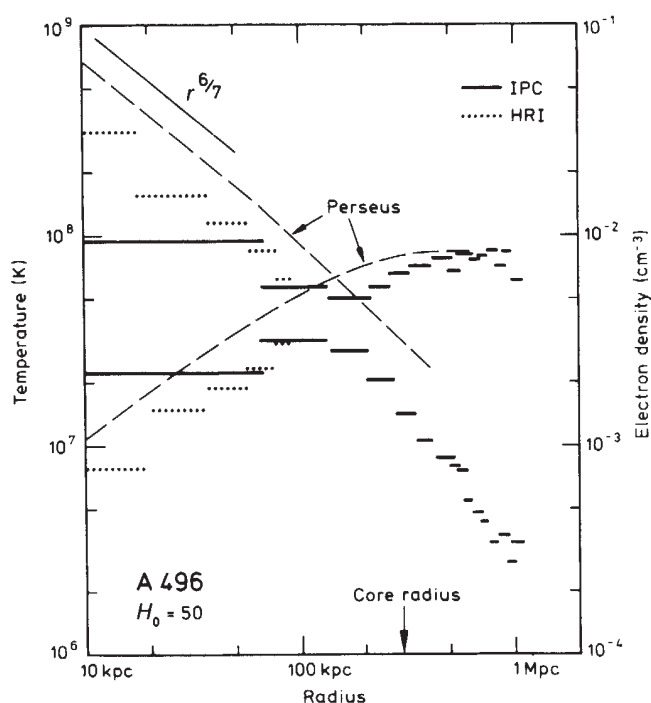


Fig. 3 Density and temperature profiles of the intracluster gas in A496²⁹. Different symbols denote the two detectors used. The profiles for the Perseus cluster²⁸ (A426) are indicated, as is the shape of the constant-pressure line cooling solution ($n \propto r^{-6/7}$).

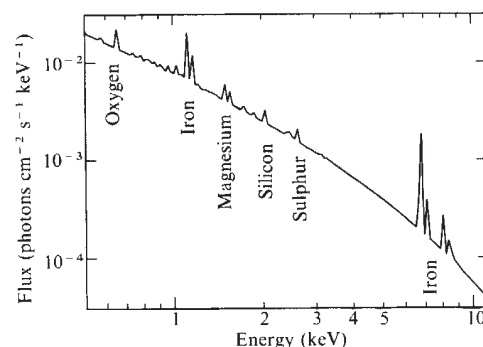


Fig. 4 Theoretical X-ray spectrum of the inner 150 kpc region of a cooling flow with $\dot{M} = 100 M_{\odot} \text{ yr}^{-1}$ at a distance of 100 Mpc. This figure was produced from a program written by H. Itoh.

ranges. When the deduced central cooling times are less than a Hubble time, the method gives estimates of the rate of mass accretion in a cooling flow, including the work done by gravity. The mass accretion rates range from values of $200\text{--}300 M_{\odot} \text{ yr}^{-1}$ for Perseus²⁸ and A496²⁹, through $20\text{--}100 M_{\odot} \text{ yr}^{-1}$ for the Centaurus (G. C. Stewart *et al.*, in preparation) and poor clusters²⁴, to $5 M_{\odot} \text{ yr}^{-1}$ for M87³⁰ (Fig. 5). More recently, Stewart *et al.*³¹ applied the technique to 36 clusters from the sample of Jones and Forman³². At least 30% of those clusters show evidence of cooling flows.

In addition to the evidence for clusters, a thermal interpretation for the soft X-ray emission observed from many elliptical galaxies^{14,15} implies a relatively short cooling time (Fig. 2). An outflow, or wind, requires an impossibly high gas production rate, whereas a cooling flow consumes only $\sim 1 M_{\odot} \text{ yr}^{-1}$, which is compatible with stellar mass loss¹⁵.

Direct evidence for cooling gas at the centres of clusters comes from X-ray spectroscopy. X-ray emission line measurements (Fig. 4) are particularly useful because they give relatively unambiguous temperature measurements and because the line strengths can be used to estimate the mass flow rates³³. The Einstein Solid State Spectrometer (SSS) is efficient for detecting the Si and S lines and the blend of Fe *L* lines that are strong in plasmas with temperatures around 10^7 K. The Einstein Focal-Plane Crystal Spectrometer (FPCS) was able partially to resolve the various contributors to the Fe *L* blend for the strongest sources and detect O VIII lines from plasma at temperatures around 3×10^6 K.

The detection with the FPCS of a strong oxygen line from the centre of the plasma halo surrounding M87 in the Virgo cluster established the presence of gas that was significantly cooler than the bulk of the X-ray emitting material, giving the first unambiguous evidence of a cooling flow³⁴. This was confirmed by broad-band IPC spectra²¹, by spectra obtained with the SSS³⁵ and by additional crystal spectrometer data³⁶. The latter shows lines from plasma at a wide range of temperatures with an emission measure distribution consistent with that expected for cooling gas. There is now evidence for low-temperature gas in the cores of half a dozen clusters (Table 1).

Broad-band spectral measurements of the integrated cluster emission obtained with proportional counters can also give clues to the presence of cooling flows, although the evidence is more ambiguous. For several clusters, multi-temperature models are required to fit the spectra³⁷⁻⁴¹. Several of these are known to have cooling flows. Although the others might be considered prime candidates for further investigation, cooling flows are not the only explanation for multi-component broad-band spectra, especially if the 'cool' component temperature is ≥ 2 keV.

The detection of optical filaments is a less direct but more easily obtainable indicator of cooling flows (see below). Filaments have been seen in the cores of many clusters that show X-ray evidence of cooling flows (Table 1). Other clusters with optical filaments do not show soft X-ray emission but the limits, when available, are not restrictive.

The optical line emission is similar to that observed from shocked gas, with [O II], H α and [N II] giving most of the emission. Ly α emission from the filaments around NGC1275 in the Perseus cluster⁴² and the central galaxy in A1795 (ref. 43) have been detected with the International Ultraviolet Explorer (IUE). Neutral hydrogen has been observed in absorption against NGC1275 with an infall velocity of 300 km s^{-1} (ref. 44).

Radial variation of $\dot{M}$

For the two best resolved and best studied cases, Perseus and M87, the deconvolution technique returns values of $\dot{M}$ that increase with radius. The increase is from 20 to $200 M_{\odot} \text{ yr}^{-1}$ out to 2 arc min ($\sim 60 \text{ kpc}$) in Perseus (Fig. 6) and from ~ 1 to $10 M_{\odot} \text{ yr}^{-1}$ out to 15 arc min ($\sim 70 \text{ kpc}$) for M87³⁰. Qualitatively, the fall in $\dot{M}$ towards the centre of the flow is revealed by the slight flattening of the surface brightness profile at small radii. That is, although the overall profile is sharply peaked and

Table 1 Evidence and references

Cluster	$\dot{M}$ ($M_{\odot} \text{ yr}^{-1}$)	X-ray surface brightness	X-ray spectrum	H α filaments
Perseus	250	28	25, 82	64, 67, 79
A496	200	29	29	67, 88, 89
M87	10	24, 31, 80, 81, 95	35-37, 95	90, 91
Cen	22	30	86	92, 93
AWM7	40	23, 24, 83, 84		
MKW3s	100	23, 24, 84		
AWM4	25	23, 24		
MKW4	20	23, 24		
A85	100	31, 32		
A262	28	31, 32		
A400	2	31, 32		
A1060	6	31, 32		
A1795	400	31, 32	3	67, 89, 94
A1983	7	31, 32		
A1991	115	31, 32		
A2029	250	31, 32	3	67
A2063	26	31, 32		
A2017	18	31, 32		
A2199	110	31, 32	3	67
A2319	75	31, 32		
A2415	15	31, 32		
A2626	10	31, 32		
A2657	36	31, 32		
SC0107-46	4	31, 32		
SC1842-63	3	31, 32		
3A0335+096	280	83	3	
A576		85	87	

thus indicative of cooling as described above, a more detailed examination shows a deficiency of flux at small radii relative to that expected in a flow with constant $\dot{M}$. Changes in the assumed cluster parameters do not alter the conclusion that mass drops out of the flow over a significant range of radius. We note that the optical filaments associated with instabilities in cooling gas often extend over similar radii.

Alternative interpretations

We have argued that there is considerable evidence for cooling flows in clusters of galaxies. The implications are that $1\text{--}100 M_{\odot} \text{ yr}^{-1}$ have accreted into a central galaxy for times exceeding $5 \times 10^9 \text{ yr}$. Therefore, a significant part, if not all, of that central galaxy is due to the flow. As these galaxies resemble most other galaxies, it might be thought that these implications are wrong.

Heating (see refs 45, 46) could prevent a cooling flow only if the heating profile exactly matched that of the cooling. Moreover, the dependence of the heating rate on the density must be particularly steep if the heating is not to make the gas more thermally unstable³⁰. It must also be equally effective in both radio-loud and radio-quiet clusters. This makes any heating proposal decidedly *ad hoc*. Any substantial non-thermal contribution to the X-ray emission is also ruled out.

Star formation according to a standard galactic initial mass function (IMF)^{47,48} at the implied rates must give many supernovae which could heat the flow^{34,49}. Perhaps the flow operates intermittently. There is evidence for A stars in NGC1275⁵⁰ in the Perseus cluster but not for earlier-type stars⁴²⁻⁵¹. Massive stars, likely to give supernovae at least, are not observed in any cooling flow galaxy. The IMF of stars from a cooling flow need not be identical to that in our galactic neighbourhood (see below). The smooth gas density profiles inferred from X-ray data argue⁴² against disturbance by an intermittent flow on time scales $\geq 10^9 \text{ yr}$.

This last point is also relevant to the duration of the flows. The smooth profiles and general consistency with cooling flow theory out to $t_c \approx 10^{10} \text{ yr}$ suggest that the flows have persisted for $5 \times 10^9 \text{ yr}$ or more. Cooling flows have been observed in most clusters that contain a single giant galaxy and that have good

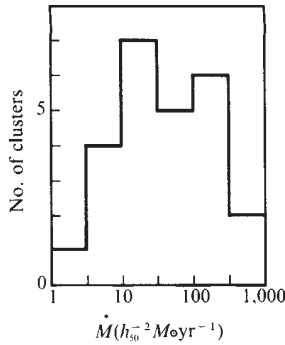


Fig. 5 Histogram of measured mass flow rates, $\dot{M}$. The bins below $10 M_\odot \text{yr}^{-1}$ are depleted by selection effects at the threshold of detectability of a flow.

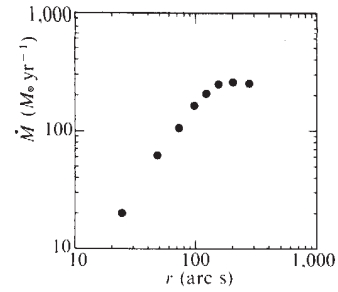


Fig. 6 The mass flow rate around NGC1275 decreases within ~ 2 arc min, from $\sim 250 M_\odot \text{yr}^{-1}$ roughly as $\dot{M} \propto r^{3/2}$. The observed optical filaments (Fig. 1) extend more than 1 arc min.

X-ray data³¹. Many of these galaxies have been found to contain optical filaments. It should be remembered that gravitational binding energy alone accounts for the observed intracluster gas temperatures of 2–10 keV (see below).

We should point out that conduction is probably inhibited in the intracluster gas by tangled magnetic fields. In particular, it has been shown that conduction cannot be occurring at the Spitzer⁵² rate in M87^{30,53}. Furthermore, the steep temperature dependence of conductivity means that it is generally either dominant or negligible, with only a small (factor ~ 2) range relevant to the X-ray data of clusters³⁰.

Theory of smooth flows

Thermal instability will generally make cooling flows very inhomogeneous (see below). Nevertheless, many of their key properties are adequately modelled by a smooth flow.

The X-ray observations of clusters of galaxies do not require any heating of the intergalactic gas other than that due to motion of the gas through the cluster potential. This is best illustrated using the parameter τ , introduced by Cavaliere and Fusco-Femiano⁵⁴,

$$\tau = \frac{\mu m_H \sigma_C^2}{kT} \quad (2)$$

where μ is the mean molecular weight and m_H the mass of a hydrogen atom. τ is a measure of the ratio of the specific kinetic energy in the galaxies to that in the gas. If the 'gravitational' histories of the gas and galaxies were identical, we would expect $\tau = 1$. However, as the galaxies are collisionless while the gas is a fluid, the two are bound to be partially separated during cluster collapse and/or mergers. Typically, the infalling gas will be stopped by the passage of a shock while the galaxies continue to fall inward, causing the gas to be less tightly bound than the galaxies and hence decreasing τ . We can, therefore, only expect $\tau \approx 1$, as is observed³². A more detailed analysis may require some additional heating of the gas, but, at present, there is no reason to believe this is the case.

We have argued that thermal conduction is negligible, so that in the absence of heating, a hydrostatic atmosphere will inevitably begin to cool substantially after about one radiative cooling time. Cooling will start in the centre of a cluster and work outward⁵⁵. Furthermore, because the cooling time is comparable to, or less than, the Hubble time in many clusters³¹, this is likely to have already happened in some.

Before the onset of cooling, there will generally be a region near the centre of a hydrostatic atmosphere over which the gas, and thus t_c , is fairly uniform. As this gas begins to cool, it is compressed by the surrounding atmosphere and increases in X-ray luminosity. The cluster luminosity thus rises, peaking at about the time when the core gas cools, almost simultaneously, to very low temperatures⁵⁵. In a smooth atmosphere this leaves

a transient pressure hole in the centre. However, thermal instability will make the onset of substantial cooling more gradual.

Once the core gas has cooled, a steady flow is established quite rapidly at the cluster centre (Fig. 7). The rate at which gas cools subsequently is determined simply from initial conditions by

$$\dot{M}(t) \equiv \left. \frac{4\pi r^2 \rho}{dt_c/dr} \right|_{t_c(r)=t} \quad (3)$$

where ρ is the gas density at radius r and equation (3) is evaluated in the initial atmosphere. This equation states simply that the gas cools after approximately one cooling time. It applies because most of the gas remains largely undisturbed until it is about to cool. The steady flow is confined to a region inside which t_c is less than the age of the system. Its extent is determined chiefly by $\dot{M}$ and the steady flow equations.

Having established that the central region of a cooling flow is well modelled as steady, we now consider its structure in more detail.

The flow velocities are typically highly subsonic (see below) and we may thus use a hydrostatic approximation. Assuming spherical symmetry, the flow equations reduce to

$$\dot{M} = 4\pi \rho v r^2 = \text{constant} \quad (4)$$

$$dp/dr = -\rho d\phi/dr \quad (5)$$

and

$$\rho v \frac{d}{dr} (H + \phi) = n^2 \Lambda(T) \quad (6)$$

where v , n , p and T are respectively the inward radial velocity, the electron density, pressure and temperature. The specific enthalpy

$$H = \frac{5}{2} \frac{kT}{\mu m_H} \quad (7)$$

and ϕ is the gravitational potential.

Note that we have ignored any addition of mass to the flow because in all known cases the steady flow is confined to well within a cluster core (see equation (12)) where the total mass loss from the galaxies falls far short of $\dot{M}$. This is in contrast to the radiative regulation model¹⁰ where all the cooling gas arises from mass loss.

If gravity is negligible, $kT/\mu m_H \gg GM/r = r d\phi/dr$, the pressure is constant and for $\Lambda \propto T^\alpha$, the temperature solution is

$$T \propto r^{3/(3-\alpha)} \quad (8)$$

α ranges from $\approx \frac{1}{2}$ for thermal bremsstrahlung to $\approx \frac{1}{2}$ for $T \approx 2 \times 10^7$ K (ref. 56), so that $6/5 \geq 3/(3-\alpha) \geq 6/7$. The density

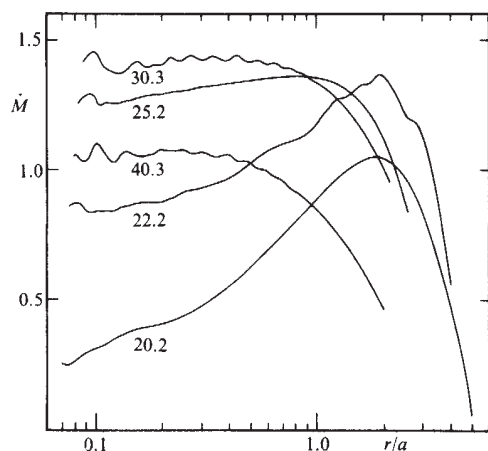


Fig. 7 Mass flow rate as a function of radius in a cooling atmosphere. This shows the onset of a cooling flow in an initially static atmosphere bound by matter distributed as $\rho_* \propto (1 + r^2/a^2)^{-3/2}$. The mass flow rate is given in arbitrary units and the time in units of the initial sound crossing time of the core. The central cooling time was initially 20. $\dot{M}$ is shown as a function of the radius shortly after the onset of rapid cooling at 20.2, and several later times (as labelled). $\dot{M}$ is independent of the radius in the region of the steady flow which has already developed by a time of 30.3. Note that $\dot{M}$ has changed considerably by 40.3 and consequently, so has the size of the region of steady flow.

varies as $\rho \propto r^{-3/(3-\alpha)}$. Constant pressure solutions apply approximately to the largest cooling flows²⁹.

When gravity is significant, $kT/\mu m_H \ll GM(r)/r$, the temperature solution always approaches a critical solution on which $kT/\mu m_H = A GM(r)/r$, for some constant A of order unity (Table 2). This behaviour is illustrated in Fig. 8. Note that, although radiative cooling drives the inflow, the temperature may fall, remain constant or rise depending largely on ϕ . In a steady smooth flow the temperature must fall eventually, but this need only occur at very small radii.

The model of a hydrostatic gas contracting due to radiative cooling is very simple and, as a consequence, the crossing time (r/v) always roughly equals the radiative cooling time throughout a flow; that is

$$r/v = Bt_c \quad (9)$$

for some $B \approx 1$. Given the temperature solutions we can estimate the density by setting $B = 1$ to get

$$n \approx \sqrt{\frac{\dot{M} k T}{\mu m_H}} \frac{3}{8\pi r^3 \Lambda(T)} \quad (10)$$

The remaining flow quantities can be calculated directly. For example, the isothermal Mach number

$$\frac{v}{\sqrt{kT/\mu m_H}} \approx \frac{1}{2kT} \sqrt{\frac{\dot{M} \Lambda}{6\pi r}} \approx 0.08 \dot{M}_2^{1/2} r_2^{-1/2} T_7^{-1} (\Lambda/5 \times 10^{-23})^{1/2} \quad (11)$$

where $\dot{M} = 100 \dot{M}_2 M_\odot \text{ yr}^{-1}$, $r = 100 r_2 \text{ kpc}$ and $T = 10^7 T_7 \text{ K}$. Table 2 gives the values of A and B for a few exact critical solutions.

The flow solutions of this section are only applicable over the region of steady flow outside the sonic point. From equation (11), the sonic point falls at $\sim 10 \text{ kpc}$ in a large cooling flow ($\dot{M} = 100 M_\odot \text{ yr}^{-1}$), and, other things being equal, its value is directly proportional to $\dot{M}$. The steady flow also breaks down outside the region where the cooling time is less than the age

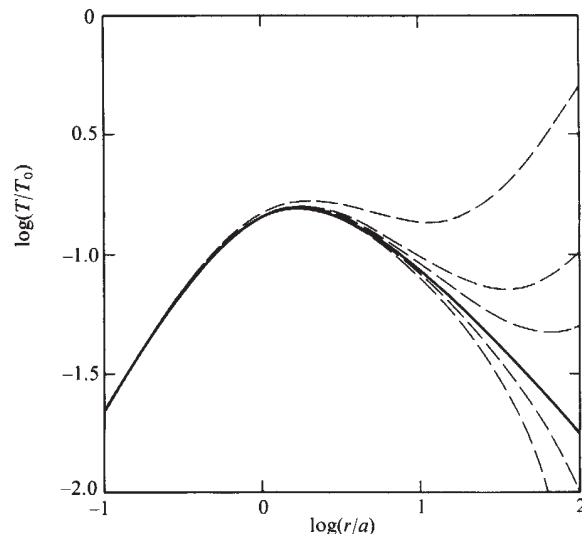


Fig. 8 Temperature versus radius for a steady cooling flow. The solutions shown are for flows in a gravitational potential $\phi = -\phi_0(a/r) \ln(r/a + \sqrt{1 + r^2/a^2})$. T_0 is defined by $kT_0 = \mu m_H \phi_0$. The critical solution (solid line) follows the potential and is the only one on which T remains finite to $r = \infty$. Solutions above this go onto the constant pressure solution, equation (8), at large r while those below terminate with $T = 0$ at some finite radius. Note that all solutions converge towards the critical solution as r decreases. (The flatness of the potential for $r < a$ results in a constant pressure solution for $r \ll a$.)

of the system, that is, for an age t , beyond radius

$$r_0 \approx \left[\frac{\dot{M} \Lambda t^2}{24\pi \mu m_H k T} \right]^{1/3} \approx 93 \dot{M}_2^{1/3} t_{10}^{2/3} T_7^{-1/3} (\Lambda/2 \times 10^{-23})^{1/3} \text{ kpc} \quad (12)$$

where $T = 5 \times 10^7 T_{7.7} \text{ K}$ and $t = 10^{10} t_{10} \text{ yr}$. This falls well within the core of most clusters.

Cowie *et al.*⁹ pointed out that angular momentum could have a substantial effect in causing a cooling flow to stagnate at some large radius. Our earlier remarks on conduction (see also ref. 57) show that the effective particle mean free paths are much less than those determined by Coulomb collisions alone⁵², in which case the ionic viscosity is negligible and cannot effectively transport angular momentum. If viscous stresses are negligible, the Reynolds number must by definition be large⁵⁸. An approximately radial flow in which angular momentum is conserved is prone to turbulence (or less dramatic shear instabilities at lower Reynolds number). If these set in, angular momentum will again be transported effectively. Thus there is a very small range, if any, of Reynolds number over which the angular momentum is able to cause a cooling flow to stagnate at large radii.

Clusters do not appear to rotate quickly so that any angular momentum in the gas is likely to be small, that is, rotation velocities

$$v_{\text{rot}}^2 \ll GM(r)/r \quad (13)$$

When angular momentum is transported effectively the flow tends to co-rotate, and the rotation is likely to remain unimportant throughout. The angular momentum of the cooled cluster gas is deposited in the remaining gas, where again it is negligible at present cooling rates. Thus treating the cooling flow as spherical is adequate for most purposes.

We note that the situation is again different in isolated elliptical galaxies where there is no surrounding medium to absorb the 'dissipated' angular momentum. In that case, the angular momentum can substantially alter the outer parts of the flow and may give rise to 'excretion' disks of neutral hydrogen¹⁵.

Table 2 Examples of exact values for $A = (kT/\mu m_H)/[GM(r)/r]$ and $B = \text{flow time/cooling time}$, for critical cooling flows

	A	B
Constant pressure solution	—	$5/(3-\alpha)$
Isothermal potential $M(r)\propto r$	$2/3$	1
Point mass potential $M(r) = \text{constant}$	$2/(6-\alpha)$	$(1-\alpha)/3$

The critical solution is that on which T remains finite as r approaches infinity. The constant pressure solution is included for comparison.

The onset and evolution of a smooth cooling flow has been discussed above (and in Fig. 7). We now consider some of the processes which alter the simple behaviour outlined there.

At present, the event most likely to disrupt a cooling flow is a cluster merger. When one cluster merges with another of comparable size or larger, its gas will not immediately be centred in the new cluster potential. Regardless of any shocks, the old central gas will still have lowest entropy, so that it must find its way to somewhere near the new cluster centre. This process inevitably causes substantial mixing and is likely to disrupt any cooling flow. Shocks caused by the merger will generally have less effect.

Apart from violent cluster mergers, a cluster potential may evolve more slowly due to continuing infall and two-body collisions in the denser parts. Although these processes are slow, they can cause substantial potential changes over a Hubble time. They may, for example, lead to the formation of a dominant central galaxy⁵⁹. Numerical calculations show that such changes do not greatly affect the evolution of a cooling flow²⁴. Because the potential change is slow, the gas is compressed adiabatically in which case the cooling time varies as

$$t_c \propto T^{-1/2-\alpha} \quad (14)$$

for $\Lambda(T) \propto T^\alpha$. Thus, changes of order unity in the cluster potential, which cause comparable changes in T , can only alter t_c by a similar amount. Slow potential changes do not dramatically affect $\dot{M}$.

As most of the gas in a cooling flow is fairly highly ionized, it is little affected by a strong central source of ionizing radiation. Numerical calculations of the ionization equilibrium in a flow have shown that the gas remains close to collisional equilibrium⁶⁰. The flow begins to be noticeably heated only when the central object is of quasar-like luminosity and Compton heating contributes strongly. Using equation (10), we find that Compton heating due to a hard central spectrum of luminosity $10^{46} L_{46} \text{ erg s}^{-1}$ overcomes bremsstrahlung cooling within a region of less than only $3 T_7^{1/2} L_{46} \dot{M}_1^{-1} \text{ kpc}$, where $\dot{M} = 10 \dot{M}_1 M_\odot \text{ yr}^{-1}$. This is well inside the region where thermal instability is important and will tend to promote that instability.

Gas cooling via collisionally-induced radiation processes is prone to thermal instability⁶¹. In a cooling flow, the growth of the instability is complicated by gravitationally induced convection^{9,62}. Gravity causes an overdense blob to move ahead of the mean flow, and so can restore it to its equilibrium position before it cools. This suppresses the instability on large scales in the outer part of the flow where the Mach number is small. At the same time, motion relative to the flow rapidly fragments most gas blobs⁶³. Thus a second effect of gravity is to fragment gas blobs repeatedly until they are, in effect, tied to the general flow.

As the flow approaches its sonic point ($r \sim 1\text{--}10 \text{ kpc}$ in a large cooling flow), thermal instability is no longer suppressed on any scale and becomes rife. This causes enhanced line emission (see below). It may also remove sufficient gas from the flow to stop the Mach number rising, causing it to saturate at some value ≤ 1 . At very small radii the remaining flow can come under the influence of a central point mass onto which it accretes. This can provide a small but significant mass flow to fuel an active nucleus¹⁵.

Detailed analysis of the thermal instability requires knowledge of the spectrum of density fluctuations in the gas which is

unavailable at present. However, as observations show that $\dot{M}$ varies with radius on all scales, we conclude that thermally unstable gas is cooling out of the flow at all radii.

Optical emission from cooling flow

Gas in a cooling flow emits optical and UV emission lines as it recombines in the temperature range $10^7\text{--}10^4 \text{ K}$. The direct luminosity in an ideal smooth flow is $10^{-3}\text{--}10^{-4}$ of the cooling luminosity. For H α in particular, the optical luminosity fraction is $\sim 10^{-4}$. Emission lines and filaments are observed in and around many central galaxies, but at a luminosity greater than that implied by single recombinations. Two effects are important here⁶⁴: repressurizing shocks in thermally unstable clumps and photoionization of cold gas by a nucleus, stars or even by the flow itself.

As a large thermally unstable blob cools, it is compressed until eventually its sound crossing time is longer than its cooling time, that is, for a size of $R \text{ kpc}$, at $\rho \sim 3 \times 10^6 R^{0.32} \text{ K}$. Such blobs cannot remain in pressure equilibrium and cool isochorically to $\sim 10^4 \text{ K}$. When cooled, their pressure may be a few hundred times less than that in the hot surrounding gas. They are repressurized by shocks which reheat the gas to 10^5 K or greater and which radiate much of the shock power above the Lyman limit. This flux photoionizes the cooling post-shock gas and leads to a much enhanced optical and UV luminosity, just as in old supernova shock waves^{65,66}. Much of the PdV work that would have appeared as soft X-radiation in a smooth flow can thereby emerge at optical and UV wavelengths. Each proton in the flow now recombines (H_{rec}) approximately five times, rather than just once. The final stages of rapid cooling are likely to lead to a sheet geometry with the cooled gas in very thin ($\ll 1 \text{ pc}$) layers. Current optical observations are unlikely to have actually resolved any of the filaments.

The mass flow rate (that passes through repressurizing shocks) is in principle determined by the emission line fluxes. Model shock calculations⁶⁶ predict line ratios and H_{rec} for various shock velocities. The line strength in $H\beta$ then gives $\dot{M}$ from

$$L(H\beta) \approx \frac{\dot{M}}{\mu m_H} \frac{H_{\text{rec}}}{5} \text{ eV s}^{-1} \quad (15)$$

A range of blob size, and thus of shock velocity, will complicate this estimate. The above estimate with $H_{\text{rec}} = 1$ should be a lower limit on $L(H\beta)$.

Photoionization by an active nucleus (in NGC1275, for example) and other sources of hard radiation (see ref. 67) generate further line radiation from the cooled and cooling gas. The radiation transfer is similar to that in post-shock gas. The geometry and, in particular, the radial distance to that gas are important factors. Unfortunately, little has yet been done on the observational comparison of line strengths and ratios over a range of radii.

Optical and UV spectra of cooling gas are potentially very important as they offer the chance of making abundance measurements. Other line diagnostics give density and pressure information. The possibility that X-ray cooling flows are dust-free⁶⁸ can be tested. In passing, we note that the IR waveband contains lines expected from the flow together with important stellar features that define the lower-mass end of the IMF of stars formed by the flow.

A cooling flow may stimulate or even create an active nucleus which is detectable at all wavelengths including the radio and X ray. Amorphous radio emission may also be created by the compression of magnetic fields and cosmic rays in the flow and in repressurizing shocks. A correlation is found between cooling flows and radio emission from a central cluster galaxy^{32,69}. It is possible that cooling flows surround many prominent radio galaxies.

Star formation

With an accretion rate of $10\text{--}300 M_\odot \text{ yr}^{-1}$ persisting for $10^9\text{--}10^{10} \text{ yr}$, the accumulated accretion flow will represent a sig-

nificant fraction of the mass of the central galaxy. The most plausible endpoint of the flow is star formation (limits on the H I content of these galaxies are many orders of magnitude too small^{70,71}). However, an initial mass function similar to the average one for the disk of our Galaxy is ruled out by the near normal colours of the central galaxies^{43,49,68}. We⁶⁸ and, separately, Sarazin and O'Connell⁷² have argued that the physical conditions in the flow would favour the formation of low-mass stars. In particular, the Jeans mass is 10–100 times lower in the high-pressure environment of the cooling flow than in the Galaxy. Although the connection between Jeans mass and the initial mass function is not understood, there is evidence that even in the Galaxy lower mass stars form in regions containing smaller, more dispersed gas clouds⁷³. The lack of detectable H I does not contradict star formation, because the collapse of the H I clouds proceeds on a short time scale.

Given the lack of plausible alternative sinks, the argument can be reversed and the X-ray measurement of $\dot{M}$ can be equated with the star formation rate near the centres of the accretion flows. Sarazin and O'Connell⁷² have synthesized colours for a population of low-mass stars and computed their effect on the colours of the host galaxy. Observations⁷⁴ may eventually constrain the parameters of the initial mass function. We note that the simple fragmentation theory of star formation does predict an endpoint in very low mass stars⁷⁵.

Cooling flows must efficiently form matter of high mass-to-light ratio. If much of the starlight in giant central galaxies is due to a surrounding cooling flow, then the flow must create elliptical-galaxy stars. Perhaps we are seeing a delayed, or continual, version of the process of galaxy formation, or maybe the stars of elliptical galaxies can be produced in a wide variety of conditions. Work on smaller flows in galaxies and at higher redshifts is very important here.

Note that the central galaxies in the two nearest large flows, in M87 and NGC3311 (A1060), are surrounded by exceptionally large populations of globular clusters^{76,77}. Gas cooling from a high temperature tends to form globular clusters, as the Jeans mass when the gas is repressurized at 10^4 K falls in the appropriate range. Using the density estimate from above, we find the Jeans mass in the repressurized gas is

$$M_J \approx 4 \times 10^6 r_1^{3/4} (\Lambda/5 \times 10^{-23})^{-1/4} \dot{M}_1^{-1/4} \sigma_{2.5}^{3/2} M_\odot \quad (16)$$

at $10 r_1$ kpc, in a cooling flow of $10 \dot{M}_1 M_\odot \text{ yr}^{-1}$, where σ_g is $300 \sigma_{2.5} \text{ km s}^{-1}$.

Conclusion

Cooling flows are an important process in the cores of many clusters of galaxies. Measured mass flow rates exceeding $10 M_\odot \text{ yr}^{-1}$ may contribute significantly to the central galaxy. Observations in the optical and neighbouring wavebands of these galaxies allow large-scale and efficient star formation to be studied. The sensitivity of the resultant population to flow parameters, such as pressure and lack of dust, may give important clues to the process of star formation. Continual star formation due to cooling flows may be quite widespread in ordinary galaxies; within elliptical galaxies, for example. The remainder of the flow that reaches the very centre of such galaxies may create a large star cluster⁶⁰ and power a central engine. The filamentary material observed in the cores of active and other galaxies may be the product of cooling flows. A possible connection with QSOs⁷² is tantalizing. The Ly α luminosity of the filaments around NGC1275 is comparable with that of some narrow-line QSOs⁴³.

Optical spectroscopy of cooling flows allows many established diagnostic techniques to be applied. Abundance studies of large volumes of free (as opposed to photospheric or stellar) gas are beginning. The quantity and distribution of optical radiation should be related to the clumpiness of the immediate intracluster gas and thus to the distribution of stars that form. Radial velocity studies should reveal the distribution of the gravitational potential in central galaxies. A direct link to the many radio structures

is not simple. Nevertheless, a power-law density distribution of surrounding density (or pressure) may be important for the propagation, or otherwise, of jets.

Cooling flows are observed in a wide range of clusters. Because some poor clusters with a central cD galaxy have large flows²⁴, it seems that the initial conditions in subclusters are such that cooling flows are common. Merging of subclusters may then occasionally turn cooling off. Cooling flows appear to be absent from those rich clusters containing two (or more) giant ellipticals³¹. These may have involved mergers of roughly equal subclusters such that any flows surrounding those giant galaxies were destroyed by mixing and heating. Distributed and unfocused cooling could continue throughout the cores of such Coma-like clusters. Einstein FPCS observations limit the cooling luminosity to be equivalent to $\leq 250 M_\odot \text{ yr}^{-1}$ in the Coma cluster itself.

If hierarchical mergers of sub-clusters lead to present-day clusters, then cooling flows may have been more common in the past, that is, at larger redshifts. It seems possible that this may be connected with the inferred evolution of many active objects, and particularly of the underlying galaxies of strong radio sources⁷⁸. Cooling flows may provide nearby examples of dissipational cluster and galaxy formation.

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ARTICLES

Re-evaluation of Greek archaeomagnitudes

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A new technique is used to determine the secular variation of the geomagnetic ancient field in Athens. The results indicate that published Greek archaeomagnitudes were seriously affected by alteration. It is shown that all intensities obtained using modifications of the Thellier method may be affected by alteration and are, therefore, suspect.

THE Thellier thermal demagnetization method¹ and modifications of it² have been used to derive the accepted data on the secular variation of the intensity of the geomagnetic ancient field near Athens. The data I obtained previously³ relied on the linearity of a plot of natural remanent magnetization (NRM) removed on heating and cooling in zero field against the partial thermoremanent magnetization (PTRM) introduced on cooling in a known field. I show here that this test can fail to detect thermally-activated alteration processes. Therefore, any geomagnetic intensities obtained with techniques that rely solely on the NRM–PTRM linearity test are suspect³.

Alteration

The linearity test is widely used⁴ but tests for alteration can probably never be tested experimentally. It is, however, possible to calculate the expected displacement of points on a NRM–PTRM plot caused by a thermally-activated alteration process⁵. This reveals that severe displacements can occur which still preserve the linearity of the plot. The resulting value for the intensity when calculated from the slope of the straight line was six times greater. Qualitatively the reason for this is easily understood: if the activation energy for the alteration mechanism is large, alteration will take place so slowly that the change during the measurement becomes unimportant. If it is small, the process of alteration will be complete early in the measurement cycle. Only if it is of the order of that for re-orientation of the magnetic moments will it be important, in which case it is difficult to distinguish from the magnetic processes.

Note that alteration affects the measurements, not so much because it changes the mineral character of the sample, that is its susceptibility, but because it produces an additional, fictitious

moment during the measurement. Consider the following hypothetical example.

A sample is being held at a temperature T_0 . Suppose that at a higher temperature T the equilibrium distribution for the moments of the individual grains differs from that at T_0 . Let us suppose, for the sake of simplification, that it is heated to T 'instantaneously'. That is, in a time $\leq 10^{-8}$ s, so that none of the moments have had time to relax to this new distribution. If the moment is initially M_0 , then after a time T it will have changed by an amount ΔM due to the (reversible) relaxation of the moments towards this new equilibrium distribution. Now suppose that during this time the sample has undergone mineral alteration which has changed M_0 irreversibly by an additional amount A . Then the error that A introduces in the total susceptibility is proportional to A/M_0 , but the error in calculating the ancient field will be of the order of $A/\Delta M$. So the effect on the susceptibility may be small, but the error produced in the intensity may be large. Therefore, trying to detect alteration by simply measuring the total susceptibility after the measurement has been made is less effective than detecting anomalous changes while the measurement is in progress.

The results presented here have been obtained using a technique which goes some way towards detecting, and even correcting for, alteration during the measurement cycle. This technique is related to an earlier one⁶ in which the sample was held at a fixed temperature in a laboratory field within $\sim 10\%$ of the unknown field. Alteration was detected from the change in slope of a graph of sample moment plotted against the logarithm of the time. The detection of mineral alteration in that method becomes difficult if alteration results in an increase in the moment.

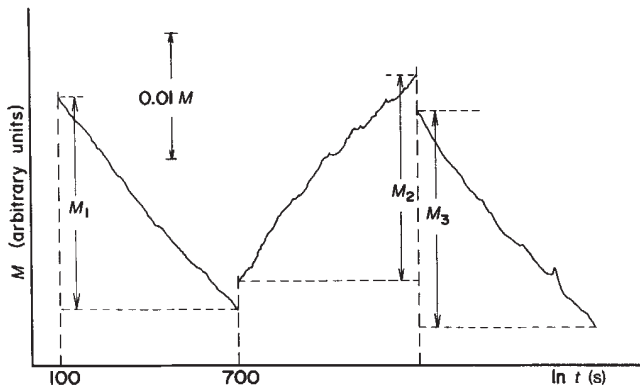


Fig. 1 The moment, M , plotted against $\ln t$ for a sample held at a temperature of 230 °C. The first step, from 100 to 700 s is demagnetization of the NRM. The second curve of increasing M is the magnetization step in a field of 50 μ T. It also begins at 100 s from the time that the field was turned on, and ends at 700 s. (The zero shift has been suppressed.) The last step is demagnetization of the viscous moment acquired during step 2. Note that the value of M at the end of this step should be $\sim 10\%$ less than that at the end of step 1. Therefore, a small amount of alteration has occurred.

Instead of monitoring the moment as a function of time in the laboratory field, the sample is held at a given temperature, in zero field for a fixed length of time. After this step, it has lost some of its moment. Then with the sample still at the same temperature a known field is applied. The ratio of moment lost to moment gained is related to a product of the ancient intensity and the logarithm of the ancient cooling rate. Mineral alteration can then be detected by performing a second demagnetization, while holding the sample temperature fixed: if alteration is still taking place it must increase or decrease the moment, which will cause an anomalous difference between the moment at the end of the second demagnetization and at the end of the first.

Theory

The magnetization of a material containing blocked superparamagnetic grains is never at equilibrium but evolves slowly with time⁷. Assuming monodomain grains, and averaging over their anisotropy, the moment is

$$M = \int_{V_{\min}}^{V_{\max}} J V n(V) N(V) dV \quad (1)$$

where J is the saturation magnetization density, V is the grain volume, $n(V)$ the distribution function, and $N(V)$ the number of grains whose volume lies between V and $V+dV$. $V_{\min}$ and $V_{\max}$ are the maximum and minimum grain volumes. All integrals over V discussed below are taken from $V_{\min}$ to $V_{\max}$.

In general, because the sample is not at equilibrium, $n(V)$ is time dependent. Allowing the volume dependence to be implicit:

$$dn/dt = (n_{\text{eq}} - n)/\tau \quad (2)$$

where n_{eq} is some equilibrium distribution function, and τ is a relaxation time, usually written

$$\tau^{-1} = c e^{-KV/kT} \quad (3)$$

where K is an anisotropy constant, and c is a rate constant of the order of 10^8 s^{-1} (refs 6, 8).

Equation (3) can be easily integrated if T is stationary:

$$\begin{aligned} n - n_{\text{eq}} &= (n - n_{\text{eq}})_{t=0} e^{-t/\tau} \\ n &= n_0 e^{-t/\tau} + n_{\text{eq}}(1 - e^{-t/\tau}) \end{aligned} \quad (4)$$

substituting equation (4) into equation (1)

$$\begin{aligned} M(t) &= \int J V N(V) n_0 \exp(-ct e^{-KV/kT}) dV \\ &+ \int J V N(V) n_{\text{eq}} (1 - \exp(-ct e^{-KV/kT})) dV \end{aligned} \quad (5)$$

The first term in equation (5) can be thought of as decay of the NRM, that is it represents the removal of the contribution of those grains whose volume is less than $V = (kT/K) \ln ct$ to the existing moment. The second term is the replacement of the lost moment with a new one.

If the original moment is a TRM produced by cooling the sample in a small field H_A , from above the Curie temperature⁷

$$n_0 = J_B V H_A / 3kT_B \quad (6)$$

where T_B is the blocking temperature for grains of volume V , and J_B is the saturation magnetization density evaluated at T_B .

The method involves three steps at a fixed temperature, T , see Fig. 1.

Step 1: Demagnetization of the NRM between time t_i and t_f :

$$H_L = 0, \text{ therefore, } n_{\text{eq}} = 0 \text{ and } n = n_0 e^{-t/\tau}$$

using equation (4)

$$n = (J_B V H_A / 3kT_B) \exp(-ct e^{-KV/kT})$$

$$M_1 = M(t_i) - M(t_f) \quad (7a)$$

$$M_1 = \int J V N(V) n_0 e^{-t_i/\tau} - \int J V N(V) n_0 e^{-t_f/\tau} \quad (7b)$$

$$\begin{aligned} M_1 &= \int J V [J_B V H_A / 3kT_B] N(V) \\ &\times [\exp(ct_i e^{-KV/kT}) - \exp(-ct_f e^{-KV/kT})] dV \end{aligned} \quad (7c)$$

it is convenient to rewrite this as

$$M_1 = -H_A F_1 J_B T / J T_B \quad (8)$$

where

$$\begin{aligned} F_1 &= F(T, t_f, t_i) \\ &= \int J^2 V^2 N(V) / 3kT [e^{-t_f/\tau} - e^{-t_i/\tau}] dV \end{aligned} \quad (9)$$

the quantity $J_B T / J T_B$ is approximately equal to $\ln cy / \ln ct$ where γ is the cooling time constant⁷ and has been taken out of the integral since $\ln ct$ does not change much between t_i and t_f .

At the end of step 1.

$$n_1 = J_B V H_A / 3kT_B \exp(-ct_i e^{-KV/kT}) \quad (10)$$

Note that $n = 0$ up to $V = (kT/K) \ln ct$ (these grains have been demagnetized).

Step 2: Magnetization in a laboratory field H_L , also between t_i and t_f : now $n_{\text{eq}} = J V H_L / 3kT$

$$\begin{aligned} M(t) &= \int J V \{ n_1 \exp(-ct e^{-KV/kT}) \\ &+ J V H_L / 3kT [1 - \exp(-ct e^{-KV/kT})] \} N(V) dV \\ M_2 &= M(t_i) - M(t_f) \end{aligned} \quad (11)$$

$$M_2 = H_L F(T, t_f, t_i) - R H_L F(T, 2t_f, t_i + t_i) = H_L [F_1 - R F_2] \quad (12)$$

where⁶

$$R = (J_B T / J T_B) H_A / H_L \cong H_A \ln cy / H_L \ln ct \quad (13)$$

A second demagnetization is used to check for alteration. The following procedure is sensitive to any alteration which is temperature- and time-dependent, for example, thermally activated processes. It depends on any change in moment due to alteration continuing during the magnetization (step 2) and a second demagnetization (step 3):

Step 3: Demagnetization in zero field.

$$n_2 = J V H_L / 3kT \{ 1 + (R e^{-t_f/\tau} - 1) e^{t_i/\tau} \} \quad (14)$$

is the distribution at the end of step 2, and the beginning of step 3. Therefore, use of equations (1), (4) and (14) yields

$$M_3 = -H_L [F_1 - F_2 + R F_3] \quad (15)$$

If $R = 1$ then after the third step, the remaining moment should

be lower than the moment at the end of step 1 by about 10%, in the absence of alteration. (Physically this may be interpreted as the result of the ancient magnetization continuing to decay, during steps 2 and 3.)

If alteration occurs equations (8), (12) and (15) may be written

$$M_1 = -RH_L F_1 + A_1 \quad (16a)$$

$$M_2 = H_L(F_1 - RF_2) + A_2 \quad (16b)$$

$$M_3 = -H_L(F_1 - F_2 + RF_3) + A_3 \quad (16c)$$

where A_1 , A_2 and A_3 are the changes in moment due to alteration in each of steps 1, 2, and 3. It is assumed that $A_1 > A_2 > A_3$ and that $A_1 < F_1 H_L < 1$.

Our objective is to compute R while minimizing the effects of A_1 , A_2 and A_3 . One way to do this is to obtain R from

$$Q = 2(M_2 + M_3 - M_1)/(M_2 - M_3) \quad (17)$$

In this way the numerator will contain $A_2 + A_3 - A_1$, the denominator $A_2 - A_3$, and the effects of alteration will tend to cancel each other; equations (16a), (16b), (16c) and (17) yield

$$R = Q - b(1 - Q/2 + Q^2/2) + c(1 + Q/2) + 1/F_1[A_1 - A_2(1 - Q/2) - A_3(1 + Q/2)]$$

where $b = F_2/F_1$ and $c = F_3/F_1$

if $t_f \gg t_i$, $c = 0.59b$

and

$$R = Q - b(0.41 - 0.84Q + 0.5Q^2) + [A_1 - A_2(1 - Q/2) - A_3(3Q/2)]/F_1$$

if $Q \sim 1$ then

$$R = Q - b(0.07) + (A_1 - A_2/2 - 3A_3/2)/F_1$$

It is expected that $A_1 > A_2 > A_3$.

Consider a specific example: if the alteration varies with time as $t^{1/2}$, appropriate for an oxidation reaction then

$$A_1 \propto (t_f^{1/2} - t_0^{1/2}), A_2 \propto (2^{1/2} - 1)t_f^{1/2}, \text{ and } A_3 \propto (3^{1/2} - 2^{1/2})t_f^{1/2}$$

and since $b \ll 1$

$$R = Q + [0.3 - (t_0/t_f)^{1/2}]A_1/F_1[1 - (t_0/t_f)^{1/2}]$$

If $t_0/t_f \sim 0.1$ the effect of alteration is negligible.

Note that alteration in the properties of the material is not as important as the fact that the properties are altering during the measurement. The change in moment due to alteration may be small compared with the moment of the sample, but important compared with M_1 , M_2 or M_3 . In other words, alteration is affecting the viscosity coefficients. Therefore, alteration that takes place before the first data point at t_0 can be safely ignored, provided that the total change in moment due to alteration is small compared with the total moment of the sample.

Experiment

The apparatus used consisted of a superconducting quantum interference device (SQUID) second-order gradiometer constructed for biological purposes. The addition of a μ -metal shield reduced the ambient field to negligible proportions, and provided a signal-to-noise ratio of better than about 3,000-to-1. A low noise level is necessary for viscosity measurements because the changes in moment are quite small.

The sample consisted of a small core 4 mm in diameter by 3 mm thick. It was held in an oven, and oriented so that its moment was parallel to the magnetometer axis. The oven was mounted on a goniometer so that the sample orientation could be changed. The oven temperature was controlled by a computer to better than 0.1 °C. The goniometer positioned the sample outside the magnetometer cryostat about 9 mm below the bottom coil of the magnetometer.

To obtain valid data, it was necessary to ensure that all ancient (those produced between the time of manufacture and the start of the measurement process) viscous moments had been

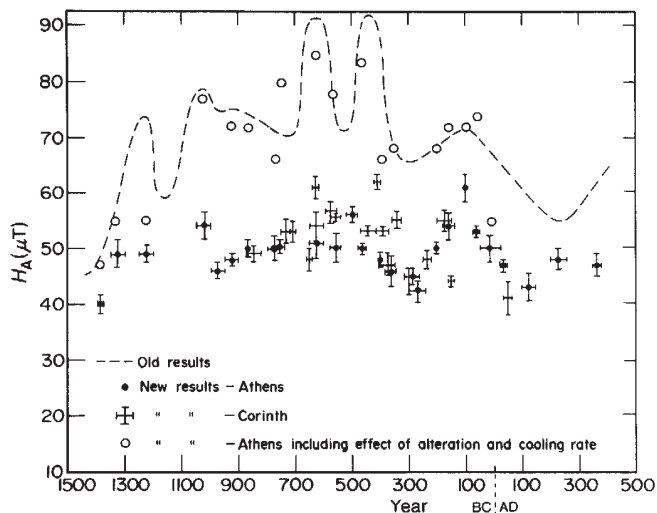


Fig. 2 Greek archaeomagnitudes obtained with the new method. The old values are indicated by the dashed line. About three-quarters of the difference is due to alteration, the remainder being the result of the fast cooling rate used in obtaining the old data. The vertical error bars represent the standard deviation determined from the total measurements for each sherd, usually a minimum of six since six temperatures were used for each sample. The horizontal bars represent the limits on the data of the sample. The dashed line represents the old data. The open circles are values of $H_L(1 + 0.1) M_1/M_3$, (the 10% increase is included to account for the difference in cooling rate). It is evident that they are close to the old data.

removed. The presence of ancient viscosity was signalled by a changing value of R . It was difficult to determine precisely to what temperature the viscous moments persisted, but for a 10-min heating period they did not appear to be removed before 220–230 °C was reached. This value agrees with a theoretical estimate obtained using the theory in ref. 7. Therefore, only data above this temperature were used.

It was important to use temperature intervals large enough to ensure that the manipulations at the preceding temperature would not have any effect. The total time at each temperature was 30 min. Experiment showed that a 25 °C temperature interval would suffice.

Table 1 Data for an Attic sherd dated 575–540 BC

T (°C)	M_1	M_2	M_3	$\frac{2(-M_1 + M_2 + M_3)}{M_2 - M_3}$	$\left(\frac{H_A}{(\mu T)}\right)$
232	-232	204	-252	0.86	43
254	-211	193	-215	0.92	46
276	-218	198	-212	1.00	50
298	-211	205	-194	1.12	56
320	-222	125	-177	1.13	56

At its present stage of development, the goniometer could not be adjusted during a run. However, on checking at the end of a run it was found that the orientation of the sample moments never changed by more than 5°.

This method isolates the effect of alteration in steps 2 and 3 through small changes in the magnetic moment of the sample. It is, therefore, a fundamental test because if the moment remains unchanged then the alteration, by definition, cannot matter. Of course, if alteration occurs only during step 1 the test will not detect it.

Using this technique the Greek material that had been used to obtain the results published in ref. 1 has been remeasured. In addition to this, results (provided by C. Williams) from a

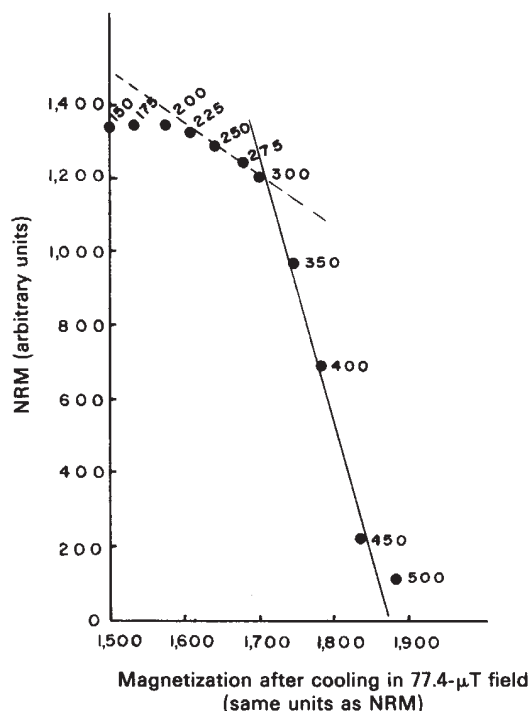


Fig. 3 Plot of NRM lost on heating in zero field and then cooling to room temperature against the total moment after heating and cooling in a laboratory field of 77.4 μT for a sample taken from a Greek sherd dated between 575 BC and 540 BC. The solid line was the preferred choice in the original report, and yielded a value for H_A of 73 μT . However, the points included have been affected by alteration. The dashed line takes in points which have not altered and yields a value for H_A of 55 μT in agreement with the data presented here.

series of sherds from the Corinth excavations have been included.

Beginning at 230 $^{\circ}\text{C}$, data were taken at 25 $^{\circ}\text{C}$ temperature intervals until the degree of alteration became too large, usually below 350 $^{\circ}\text{C}$. The ancient field was calculated from the value of R assuming that $\ln \gamma = \ln c t_f$. Since $t_f = 600$ s the cooling time for the pottery which would correspond to $\gamma = 600$ s is roughly 24 h. The value of R , in turn, was obtained from M_1 , M_2 and M_3 using equation (17) and letting $R = Q$. The results are shown in Fig. 2.

Discussion

The new values are considerably lower than the old. Some of this discrepancy can be traced to a cooling rate effect as the samples were cooled very rapidly (in about 100 s) in the old experiments. However, this only accounts for a correction of about 10% of H_A . The major reason for the discrepancy is alteration. This is apparent if the ratio M_1/M_3 at the highest temperature (~ 350 $^{\circ}\text{C}$) is plotted for each sample. The data from this temperature would be most affected by alteration.

M_1/M_3 is also equal to the ratio of NRM lost to PTRM gained in the modified Thellier method.

A detailed comparison of the old results from one of the sherds with those obtained using this new technique confirms this conclusion: the results for the new method are shown in Table 1 for a sherd dated 575–540 BC. The ratio of M_3 to M_2 is a measure of the degree of alteration, because alteration will affect M_3 and M_2 in opposite ways. This ratio becomes progressively larger as the temperature increases. At the maximum temperature employed, 320 $^{\circ}\text{C}$, the difference between $|M_2|$ and $|M_3|$ has become about one third of $|M_1|$. If this is taken to be a measure of the alteration, this is now so severe that the correction procedure we are using becomes questionable. This indicates that the results obtained above this temperature include an increasing contribution from alteration, and that this leads to an overestimate of the ancient field intensity. The original NRM–PTRM plot is shown in Fig. 3. In fact, using the points between 200 and 350 $^{\circ}\text{C}$ to obtain a value for H_A gives 0.55 μT , in good agreement with the value in Table 1, bearing in mind the need to correct for a fast cooling rate. However, in treating the original data the points above 300 $^{\circ}\text{C}$ yielded a better straight line, and according to the usual practice these were preferred. Thus, choosing the ‘best’ straight line gave the wrong answer.

The test we have used for alteration is fundamental: in effect it monitors the moment of the sample at the measurement temperature. A return to a lower temperature to check the susceptibility is unsatisfactory because a completely different set of grains is involved, and the change due to alteration is a small fraction of the total susceptibility.

It is easy, however, to employ a variant of this test in the Thellier method. All that is required is a three-step heating procedure. First, heat to a given temperature and cool in zero field; this measures moment lost. Second, heat and cool to the same temperature in the lab field. This measures moment gained. Third, heat and cool again in zero field.

Because the NRM continues to decay during steps 2 and 3, the moment at the end of the third step should be slightly lower than that at the end of the first if no alteration has occurred. (The exact difference must be calculated, of course.) Needless to say it is necessary to carefully control the time and temperature for each step.

Conclusions

This new technique makes it possible to detect the mineral alteration on heating with greater sensitivity than previously possible. It may be possible to correct for a small degree of alteration although the degree to which alteration has occurred before measurement remains an open question. Finally, it should be noted that the original data were reproducible and self-consistent. Thus the reproducibility and self-consistency of experimental results while comforting are no guarantee of accuracy. It is dangerous to rely solely on these criteria.

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Resolution of recombination intermediates generated during yeast mating type switching

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Interchromosomal gene conversion between alleles has been shown in yeast frequently to be associated with the recombination of flanking genetic markers. Although this also holds true for gene conversion between two alleles of the yeast mating-type (MAT) locus, initiated by the homothallic switching system, we find no evidence that crossing-over ever accompanies gene conversion between the non-allelic HMR and MAT genes when initiated by this same homothallic switching system.

THE alternative alleles of the mating type locus, *MATa* and *MAT α* , confer an *a* or α mating phenotype, respectively, on *Saccharomyces cerevisiae* cells. Cells containing the homothallic gene (*HO*) interchange between *a* and α cell types as often as every generation in a highly regulated pattern of cell lineage¹⁻³. This process of mating-type switching involves the substitution of genetic information copied from either the *HML* or the *HMR* locus for the allele residing at *MAT* (Fig. 1)⁴⁻¹⁰. The mating-type information residing at the *HML* and *HMR* loci, called cassettes, behave differently from that residing at the *MAT* locus in two important aspects. First, the *HML* and *HMR* information is kept unexpressed by the action of at least four different loci, variously known as *MAR* (ref. 11), *SIR* (ref. 12) or *CMT* (ref. 13); mutations in any one of these genes allow expression of the *HML* and *HMR* loci. Second, the *HM* loci in wild-type strains do not switch; they only act as donors of genetic information for transposition into *MAT* (ref. 5) (see refs 14-17 for reviews).

Current models for recombination postulate the generation of a local region of heteroduplex structure formed by two interacting homologues of DNA¹⁸⁻²¹. Gene conversion is a genetic phenomenon believed to be due to correction of the mismatched region of heteroduplex DNA. Resolution of the crossed-strand (Holliday) joint in meiosis is postulated to result in recombination of flanking markers in about 50% of cases. This expectation arises because the four-strand-stage Holliday structure can isomerize to allow reciprocal recombination accompanying the gene conversion event^{20,21}. Recent studies have provided circumstantial evidence for the suggestion that mating-type gene transposition also occurs by the general process of gene conversion^{22,23}.

Three properties, however, make mating-type switching quite distinct from the standard gene conversions observed at many other loci in yeast and other fungi. The molecular basis for the high efficiency^{17,24} unidirectionality²⁴⁻²⁸ is now known. The present report addresses the resolution of the *MAT* switching intermediate which occurs almost always without recombination of flanking markers. This is in contrast to other loci studied where gene conversion events are associated with crossing-over in as many as 50% of events²⁹.

There are several plausible reasons for the absence of crossing-over during the mating-type switching event: (1) *MAT* switching may occur by diffusible replicas of the *HM* donor loci; (2) *MAT* switching is usually an intrachromosomal event and intrachromosomal gene conversions are known to be unassociated with crossing-over in both the mitotic³⁰ and meiotic^{31,32} cycles. In such cases the extent of sequence homology between the interacting segments may be a limiting factor. (3) Specific mating-type system features such as *MAR/SIR* control³³, autonomous replication sequences (*ARS*) or other sequences

flanking *MAT* and the *HM* loci may dictate resolution of the switching intermediate without crossing-over. Some of these possibilities are tested in the results presented here.

Interchromosomal *MAT* to *MAT* transfer

We investigated whether there was an association of crossing-over of the closely linked flanking markers with *MAT* to *MAT* transposition between allelic sites. To do this we needed a diploid strain that could satisfy three criteria: (1) homothallic switches at *MAT* must be the result of sequences donated from the other *MAT* allele, not one of the usual *HML* or *HMR* donor loci; (2) the *MAT* loci must not be subject to multiple switches; (3) the flanking markers must be genetically marked.

To ensure that the switching event was interchromosomal and from one *MAT* allele to another, we constructed strains in which the normal donor loci *HML* and *HMR* were deleted (*hml Δ* and *hmr Δ*). To ensure that switching would occur only once in one of the *MAT* alleles, we used as donor allele an inconvertible allele of *MATa* which switches only rarely, *MATa-inc* (ref. 34). The defect in *MATa-inc* is known to lie in that part of the cassette that can be transposed³⁴⁻³⁶. The *mata* allele used is defective in the *MATa* function required to shut off switching in *a/a* cells, but is an efficient substrate for switching. The flanking regions were marked with the *cry1* and *thr4* mutations. The *CRY1* marker is located about 2 centimorgans proximal and *THR4* is situated about 20 centimorgans distal to *MAT* (ref. 37). These map distances are based on meiotic recombination frequencies; mitotically these markers recombine at frequencies $<1 \times 10^{-4}$. The experiment involves mating cells of strain K465 (*hml Δ cry1 mata THR4 hml Δ ho*) with cells of strain K467 (*hml Δ CRY1 MATa-inc thr4 hmr Δ HO*), allowing them to switch to *MATa-inc/MATa-inc* and scoring whether the switched strains have recombined or retained the parental configuration of the *cry1* and *thr4* alleles (see Table 1 for complete genotypes). *MAT* to *MAT* interchromosomal transpositions occur efficiently at rates equivalent to those from the *HM* loci (data not shown).

Thirty zygotes generated by mating cells of strain K465 with cells of K467 were allowed to grow to form colonies. The zygotic progeny were subcloned to obtain pure cultures derived from a single switching event. Two subclones from each zygotic progeny were analysed by tetrad analysis to ascertain the linkage between *CRY1* and *THR4* markers. The *MATa-inc/MATa-inc* cells were sporulated by the *kar1* procedure³⁸. At least 10 asci from each subclone were dissected. Each subclone had the *MATa-inc/MATa-inc* genotype and segregated 2 *CRY1*:2 *cry1* meiotic products. Linkage of *cry1* and *thr4* was determined and showed that, while 34 subclones had the parental configuration, 12 had recombined (*cry1, thr4/CRY1, THR4*): 1 homozygous *thr4/thr4*:4 homozygous *THR4/THR4*. Thus, a significant pro-

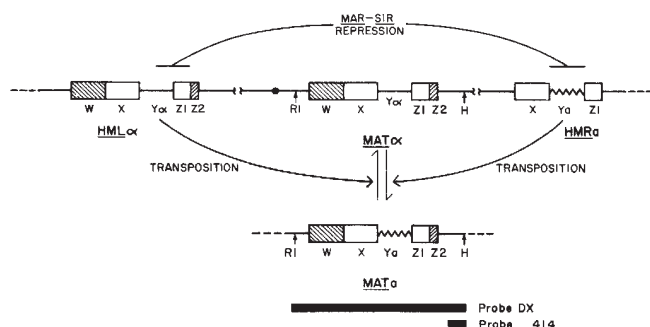


Fig. 1 Arrangement of *HML*, *MAT* and *HMR* loci on chromosome III and the process of mating type switching. The *MAT* and the *MAT* α alleles differ by an allele-specific DNA substitution: *Y* α is a unique 642-bp and *Y* α is a unique 747-bp segment. Each of the *HML*, *HMR* and *MAT* loci carry the *X* region (707 bp) to the left and the *Z1* region (239 bp) to the right of *Y* region. *HML* and *MAT* share two additional homologies: the *W* region (723 bp) and the *Z2* region (88 bp)⁴⁹. *HML* and *HMR* are located on opposite arms at least 100 kb away from *MAT*⁵⁰⁻⁵². *MAT* α and *MAT* each code for two transcripts that are divergently transcribed from the *Y* region to the outside^{53,54}. *MAT* switching occurs by substituting information residing at *MAT* with the information copied from *HML* or *HMR* by a unidirectional transposition process⁴⁻¹⁰. The DNA sequence replaced from *MAT* is apparently lost. The *MAR/SIR* control keeps the *HM* loci unexpressed and also does not allow them to switch. The solid dot represents the centromere. R1, *EcoRI* endonuclease site; H, *HindIII* site. The DX probe sequence is comprised of about 3.7-kb *EcoRI*-*HindIII* *MAT* α sequence, while probe 414 is terminal ~400 bp centromere-distal *MAT* sequence derived from the *XhoI* linker insertion mutant. The physical map is not drawn to scale.

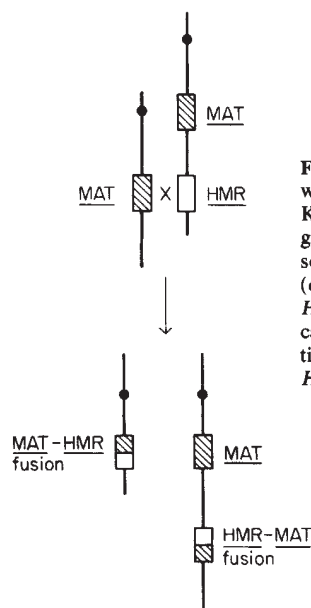


Fig. 2 Crossing-over associated with *mata* to *MAT* α switching in K604 × K465 zygotic progeny should generate two recombinant chromosomes, one containing the *MAT* (centromere-proximal region)-*HMR* (distal region) recombinant cassette (that is, Hawthorne's deletion⁴²) and the other containing *HMR* (proximal)-*MAT* (distal) cassette (the *SADI* mutation⁴¹).

portion of *MAT* switches are associated with recombination of flanking markers, a result indicating a physical interaction between the donor and the recipient during switching. No switching and recombination was observed in *ho* cells (data not shown).

Interchromosomal *HMR* to *MAT* transfer

In contrast to the experiments described above, *MAT* switching is usually an intrachromosomal gene transfer event where the genetic information is transmitted from the donor *HM* loci to *MAT*. The question then arises whether the absence of crossing-over in the *MAT* switching system is simply a consequence of the fact that *MAT* switching is usually an intrachromosomal event. If so, we should find crossing-over when the information

Table 1 Genotype of strains used

K465	<i>hml</i> Δ^* <i>mata</i> <i>hmr</i> Δ <i>ho</i> <i>MAR</i> ⁺ <i>cry1</i> <i>hix3</i> <i>tyr7</i> <i>met13</i> <i>leu2</i> (?) <i>MAL2</i>
K467	<i>hml</i> Δ <i>MAT</i> α - <i>inc</i> <i>hmr</i> Δ <i>HO</i> <i>MAR</i> ⁺ <i>thr4</i> <i>trp1</i> <i>metx</i> <i>urax</i> <i>leu2</i> (?) <i>MAL2</i>
K581	<i>hml</i> Δ <i>MAT</i> α <i>hmra</i> :: <i>LEU2</i> <i>HO</i> <i>MAR</i> ⁺ <i>leu2</i> <i>trp1</i> <i>hix3</i>
K496	<i>hml</i> Δ <i>mat</i> Δ <i>hmr</i> Δ <i>HO</i> <i>MAR</i> ⁺ <i>thr4</i> <i>his4</i> <i>trp1</i> <i>leu2</i> <i>urax</i> <i>MAL2</i>
K526	<i>hml</i> Δ <i>mat</i> Δ <i>hmr</i> Δ <i>HO</i> <i>mar1</i> <i>thr4</i> <i>leu2</i> <i>trp1</i> <i>urax</i> <i>MAL2</i>
K582	<i>hml</i> Δ <i>MAT</i> α <i>hmra</i> :: <i>LEU2</i> <i>ho</i> <i>mar1</i> <i>leu2</i> <i>trp1</i>
K597	<i>hml</i> Δ <i>MAT</i> α <i>hmra</i> :: <i>LEU2</i> <i>ho</i> <i>mar2</i> <i>leu2</i> <i>trp1</i> <i>his1</i> <i>lys2</i>
K599	<i>hml</i> Δ <i>mat</i> Δ <i>hmr</i> Δ <i>HO</i> <i>mar2</i> <i>thr4</i> <i>leu2</i> <i>his1</i>
K604	<i>hml</i> Δ <i>MAT</i> α - <i>inc</i> <i>HMR</i> α <i>HO</i> <i>leu2</i> <i>ade6</i> <i>lys2</i> <i>ura1</i> <i>trp1</i> <i>met</i> (?) <i>mal</i> ⁻

* Δ represents deletion of a particular locus. The procedures for construction of deletions of *HML*, *MAT* and *HMR* have been described elsewhere^{36,47}. The *hmra* :: *LEU2* allele carries a deletion of 70 bp that spans the *Y/Z* junction, ending 9 bp into *Z1* into which the 2.2-kb *SalI*-*XhoI* *LEU2* fragment was inserted. This construction was first made at *MAT*²⁴. The internal fragment contains a part of the *X* region, all of the *Y* region (including the *LEU2* insert) and part of the *Z1* region. The fragment was introduced into *HMR* by transformation, using a linear piece of DNA, and selection for the *LEU2*⁺ marker⁴⁸.

is transposed from the *HM* locus residing on one chromosome to the *MAT* locus situated on the homologue.

To test this prediction, the sterile and sporulation-proficient progeny of K604 (*hml* Δ *MAT* α -*inc* *HMR* α *HO*) × K465 (*hml* Δ *mata* *hmr* Δ *ho*) diploid zygotes were analysed by tetrad analysis. These result from interchromosomal gene transposition where *mata* is changed to *MAT* α since the original *MAT* α -*inc*/*mata* K604 × K465 zygotic cells express an α phenotype and will not sporulate. The *MAT* α -*inc*/*MAT* α cells cannot switch further¹⁴. If crossing-over were associated with switching, we would expect diploid cells in which both recombinant products (Fig. 2) should be present. The chromosome bearing the *HMR*-*MAT* fusion cassette (known as *SADI*) contains a duplication of the chromosome region between the *MAT* and *HMR* cassettes³⁹⁻⁴¹ while the *MAT*-*HMR* fusion cassette reciprocal structure (known as Hawthorne's deletion) is deleted for the corresponding segment^{42,43}. Haploid cells bearing the Hawthorne deletion die⁴². Consequently, events in which crossing-over is associated with switching should generate 2 live:2 dead meiotic products in each ascus.

A total of 26 independent *MAT* α -*inc*/*mata* to *MAT* α -*inc*/*MAT* α switches were analysed by tetrad analysis. At least five asci from each switching event were dissected. None segregated 2 live:2 dead, indicating the absence of crossing-over associated with interchromosomal gene transposition. Spore viability in these dissections was high (~86%). Furthermore, the *MAL2* marker, which is tightly linked to *HMR* (<1 centimorgan)³⁷, segregated 2⁺:2⁻ and in pooled data, parental linkage of *MAT* allele to *MAL2* (and therefore the tightly linked *HMR*) allele was maintained. Both observations demonstrate that the parental configuration of chromosome III markers was maintained during switching. Therefore, the switched cells must have resulted from proper interchromosomal *HMR* to *MAT* transpositions. Such switches were *HO* dependent (data not shown).

The results presented thus far demonstrate that interchromosomal transpositions between allelic sites (*MAT* to *MAT*) are associated with recombination of flanking markers whereas interchromosome and intrachromosome transpositions (presented below) from the *HMR* locus to *MAT* are not. Therefore, inter- versus intrachromosome difference alone is not sufficient to explain this phenomenon. However, some other special feature may have been selected into this system, because associated crossing-over during *HM* loci to *MAT* transposition would result in lethal deletions. This outcome is predicted from the three cassettes being arranged in the same orientation on chromosome III (Fig. 1). The following studies test whether this property is dictated by the *MAR/SIR* control which represses

the expression and ability of the *HM* loci to switch. The *MAT* locus is not known to be regulated by this control and therefore *MAT* to *MAT* transpositions may resolve with crossing-over. The following studies test whether the *MAR/SIR* control affects the resolution of the switching intermediate constructed during *HMR* to *MAT* transposition.

Effect of *MAR/SIR* control

To test whether *MAR*⁺ control affects recombination of flanking markers a *MAR*⁺ strain K581 with the genotype *hmlΔ MATα THR4 hmra-inc::LEU2 ho* was constructed. The *hmra-inc::LEU2* allele contains a deletion of ~70 base pairs (bp) spanning the Y/Z boundary. In place of the deleted sequence a 2.2-kilobase (kb) fragment of DNA containing the *LEU2*⁺ gene was inserted by recombinant DNA techniques. As the deletion removes the site (YZ junction) where the *HO*-dependent endonuclease cleaves *MAT* (ref. 24), this construction acts as a non-switchable (*inc*) allele when transposed into the *MAT* locus. To provide the *HO* gene function required for switching, the K581 cells were mated to cells of K496 (*hmlΔ matΔ thr4 hmrΔ HO*). Strains containing the deletion of *MAT* mate as α cells and the *MATα/matΔ* diploids behave as α cells and are therefore expected to switch. Switches of zygotic cells and their progeny from α (*MATα*) to a (*mata*) were monitored in a cell lineage by the procedure described previously which examines switches at the single cell level². Sister cells of each switched pair were grown separately into colonies and analysed.

Recombination of flanking markers during *HMR* to *MAT* transposition in this experiment would fuse *MAT* with *HMR*, resulting in loss of the acentric DNA piece containing the *THR4*⁺ marker. Cells with such recombinations should thus exhibit a *Thr*⁻ phenotype. The resulting switched *mata-inc::LEU2* allele should not switch further because of the *inc* defect. Because switches occur in pairs of cells, it is thought that a newly switched allele is replicated and segregated into two sister cells³. If so, a crossing-over event should generate both cells of a switched pair with the *Thr*⁻ phenotype.

The competent cells were observed to switch with an efficiency of 40%. We find that diploids frequently switch at a slightly reduced frequency (39–50% compared with ~76% in haploids)²⁵. A total of 136 pairs and 16 single switched cells were tested for threonine requirement. None of the pairs included both *Thr*⁻ sister cells (Table 2). However, 10 pairs unexpectedly produced 1 *Thr*⁺:1 *Thr*⁻ cells.

Analysis of DNA isolated from the 1 *Thr*⁺:1 *Thr*⁻ pairs provided an interesting explanation. Figure 3 shows the analysis of six pairs. The transposition of *LEU2* insert from the *hmra::LEU2* cassette into *MAT* should generate a new 6.4-kb *Hind*III fragment with the concomitant loss of 4.3-kb *MATα* fragment. All the *Thr*⁺ cells carry the predicted 6.4-kb fragment. However, four among six *Thr*⁺ switched cells (lanes e, f, k, o) carry an additional cassette (7.14 kb) running slightly above the 7.1-kb *hmra::LEU2* fragment. Subsequent experiments (described below) showed it to be a *SAD1*-like rearrangement, which results from a recombinant cassette containing *HMR* proximal and *MAT* distal sequences, and thus has a tandem duplication of sequences between *MAT* and *HMR*⁴¹. All *Thr*⁻ switched cells instead possess the *MAT*-*HMR* fusion (Hawthorne's deletion) of predicted size 6.36 kb; accordingly, the *hmra::LEU2* cassette is missing.

To confirm the identity of the presumed Hawthorne's deletion in *Thr*⁻ and the *SAD1* cassettes in *Thr*⁺ switched cells, the DX probe was washed off the nitrocellulose filter used for Fig. 3 and the filter was rehybridized with probe 414. This probe comprises ~400 bp of the centromere distal end of the *MAT* *Hind*III fragment (see Fig. 1) and is predicted to be missing in a strain containing the Hawthorne's deletion, but to be present on *Hind*III fragments bearing the *MAT* and the *SAD1* cassettes. The predicted results were obtained (Fig. 4). In addition, the extent of hybridization of the probe to *Hind*III fragment containing the *SAD1* cassette in each case was constant and was equivalent to that seen with the *MAT* containing fragment,

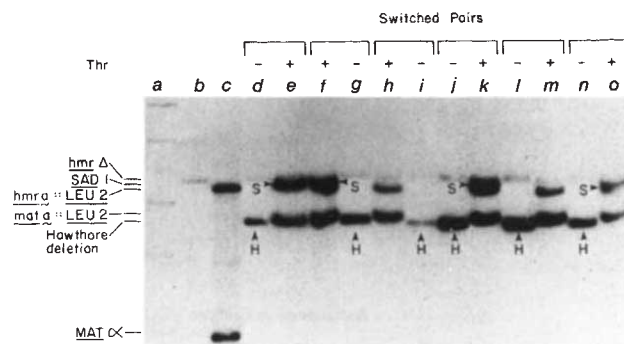


Fig. 3 Southern blot analysis⁵⁵ of *Thr*⁺:*Thr*⁻ pairs of switched cells of K581 × K496 zygotic progeny. Lane a, size markers; lane b, K496; lane c, K581; lanes d–o represent six different *Thr*⁺:*Thr*⁻ pairs. H, Hawthorne's deletion cassette; S, *SAD1* cassette. The strain K496 carries sequences homologous to the DX probe on a smaller 1.7-bp *Hind*III fragment representing the *matΔ* allele from which the whole cassette sequence has been deleted. Because of its small size this fragment ran off the gel presented. DNA was prepared from stationary phase grown cultures⁵⁶. ~1 μg DNA from each strain was digested with the *Hind*III endonuclease, fragments were separated by agarose gel electrophoresis and then transferred to a nitrocellulose filter⁵⁵. The filter was probed with ³²P-labelled pBR322 plasmid containing the DX (Fig. 1) sequence and subjected to autoradiography⁸. As the DX probe has extensive homology to *HML*, *MAT* and *HMR* sequences, fragments bearing those cassettes can be visualized.

indicating stability of the chromosome possessing the *SAD1* rearrangement. Based on these results it seems that most (6 among 10 tested) *Thr*⁻ cells do not result from direct fusion of *MAT* with *HMR* but are instead a consequence of an unequal sister chromatid exchange, with recombination occurring between different cassettes. This generates one chromatid carrying the Hawthorne's deletion and the other the *SAD1* rearrangement (see Fig. 5). These recombination products are distributed into two sister switched cells.

The additional *Thr*⁺:*Thr*⁻ pairs presented in Table 2 are also not a result of recombination of flanking markers as these events would generate *Thr*⁻ pairs of switched cells. Therefore, we conclude that there is very little, if any, recombination of flanking markers during *HMR* to *MAT* transposition in standard *MAR*⁺ strains. In addition, the *hmra-inc::LEU2* allele acts as a good donor and we presume that the cassette fusions do not result because the donor allele contained an insert of 2.2-kb *LEU2* DNA. Note that the *MAR/SIR* control may not be operative at the *hmra-inc::LEU2* allele. Should this control dictate resolution, we expected recombination of flanking markers in nearly 50% of switching events. This was clearly not observed. Haber *et al.*⁴⁴ have shown that intrachromosomal transpositions between duplicated *MAT* genes placed at *MAT* occur efficiently and only 1–5% switches accompany reciprocal recombination. In the light of our results such recombinations may also be due to uneven exchanges.

To test the *mar1* and *mar2* effect on resolution, an experiment similar to the one presented above, but with *mar1* mutant strains, was conducted. The switching efficiency of competent cells of K582 (*hmlΔ MATα THR4 mata-inc::LEU2 ho mar1*) × K526 (*hmlΔ matΔ thr4 hmrΔ HO mar1*) zygotic progeny was observed to be 40% (24 among 60 tested). Among a total of 124 pairs and 53 single switched cells assayed, 9 pairs contained 1 *Thr*⁺:1 *Thr*⁻ cells, one pair were both *Thr*⁻ and five single cells were *Thr*⁻. Southern blot analysis (data not shown) of the pairs containing the *Thr*⁻ switched cells is summarized in Table 2. Only one pair, in which both members were *Thr*⁻ and both contained the Hawthorne's deletion, may have resulted from switching associated with crossing-over.

In the same way, the effect of *mar2* on the resolution of the recombination intermediate was investigated using K597 (*hmlΔ*



Fig. 4 Identification of Hawthorne's deletion and *SAD1* cassettes present in K581×K496 switched Thr⁺:Thr⁻ pairs. The same nitrocellulose filter used for the experiment presented in Fig. 3 was immersed in 0.1% Sarcosyl at 80°C for 10 min to wash off the DX probe. The filter was rehybridized with ³²P-labelled probe 414. Lanes as in the legend to Fig. 3.

MATα THR4 hmra-inc::LEU2 ho mar2 × K599 (*hmlΔ MATΔ thr4 hmrΔ HO mar2*) zygotic progeny. Among 58 pairs of switched cells tested, only one 1 Thr⁺:1 Thr⁻ pair was produced.

Discussion

We have shown that efficient recombination of flanking markers occurs during *MAT* to *MAT* interchromosomal gene transposition mediated by the mating-type switching mechanism. In contrast, both inter- and intrachromosomal *HMR* to *MAT* transpositions occur without crossing-over. This lack of recombination is not regulated by the *MAR/SIR* control. Usually *HML* and *HMR* loci act as efficient donors for *MAT* cassette switching. The results presented here demonstrate that the *MAT* cassette may act as an efficient donor as well.

The low frequency (~1%) of fusions between *MAT* and the *HM* loci found in *HO* cells during intrachromosomal gene transfer events has previously been used to argue for recombination of flanking markers, and hence gene conversion, as the mechanism for transposition²². Our results demonstrate that these fusions instead may be arising from unequal exchanges between different cassettes (this paper) or because of the secondary consequence of the double-strand DNA break found at *MAT* in Mar⁺ (ref. 24) and also at the *HM* loci in Mar⁻ cells²⁸. Therefore, the best published result which is consistent with the idea of gene conversion as the mechanism for cassette transpositions is the observation of efficiently generating wild-type *MATα* recombinants from two different mutant *a* cassettes, one residing at *MAT* and the other at *HMR* (ref. 23).

The observation that *MAT* switching always occurs in pairs of cells^{2,3} implies that switching occurs before or at the time of *MAT* DNA replication. In contrast, the sister chromatid exchanges observed here must occur in the G₂ phase of the cell cycle. These events are observed at a reasonably high frequency and are therefore likely to be mediated directly or indirectly by

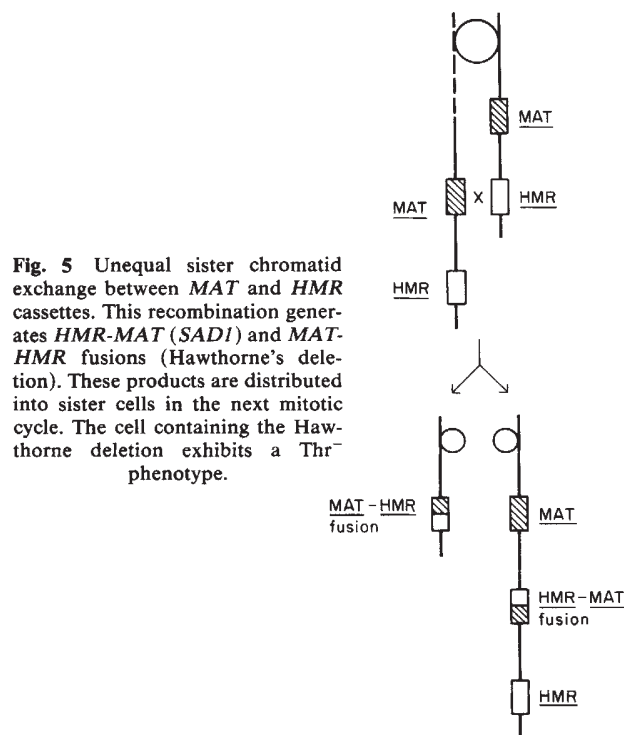


Fig. 5 Unequal sister chromatid exchange between *MAT* and *HMR* cassettes. This recombination generates *HMR-MAT* (*SAD1*) and *MAT-HMR* fusions (Hawthorne's deletion). These products are distributed into sister cells in the next mitotic cycle. The cell containing the Hawthorne deletion exhibits a Thr⁻ phenotype.

the switching mechanism. We may imagine that regions of the chromosome bearing the *HM* loci may replicate before the switching event. Following interconversion, *MAT* or the *HM* loci may exist in a configuration allowing recombination with the other cassettes (for example, unfilled gaps or nicks in the DNA). We realize that another interpretation of the results could be that switching occurs after *MAT* DNA replication; however, in this model, to explain the pairs rule, one must further postulate that when one chromatid switches the sister must always follow suit.

Why is there no recombination of flanking markers during *HM* loci to *MAT* transposition? Is this a general property of an intrachromosomal gene conversion? Recent studies on duplicated *his4* (ref. 30) elements in mitotically growing yeast cells and on duplicated *his3* (ref. 32) and *leu2* (ref. 31) elements in meiotic cells also show a lack of crossing-over associated with intrachromosomal gene conversion. We have shown here that not only intrachromosomal but interchromosome transpositions from the *HMR* locus to *MAT* occur without crossing-over. Therefore, the difference between inter- and intrachromosome transposition does not in itself explain the lack of crossing-over in the *MAT*-switching system. The DNA sequences around the *HM* and/or the *MAT* loci may control resolution. Additionally, the extent of sequence homology between different cassettes may not be sufficient to allow isomerization of Holliday junctions. The same reason may also explain the lack of crossing-over associated with intrachromosomal gene conversions of *his4*, *his3* and *leu2* duplicated elements. This property has rather profound biological implications, as associated crossing-over during gene conversions between non-allelic sites will cause deleterious deletions, inversions and translocations.

Based on studies of mitotically growing cells, Esposito⁴⁵ has concluded that spontaneous gene conversions in yeast result from the repair of heteroduplex DNA formed at the two-strand stage in the G₁ phase of the cell cycle and that Holliday junction resolution occurs by DNA replication. Note that the autonomously replicating sequences (*ARS*) are present at sites closely flanking the *HM* loci⁴⁶. These *ARS* sequences may resolve the switching intermediate by DNA replication in a way precluding crossing-over of flanking markers. It is interesting

Table 2 Rearrangements in Thr⁺:Thr⁻ switched pairs

Pair	<i>MAR</i> ⁺ * (K581×K496)	<i>mar1</i> (K582×K526)
Hawthorne's deletion: <i>SAD1</i>	6	4
Hawthorne's deletion: standard chromosome	3	2
Hawthorne's deletion: Hawthorne's deletion	0	1
Loss of chr.III: standard chromosome	1	1
Loss of chr.III: loss of chr.III	0	1

* A total of 136 pairs in the *MAR*⁺ and 124 pairs in the *mar1* experiment were tested for threonine requirement.

that ~10% of *MAT* to *MAT* interchromosomal transpositions are associated with homozygosity of the distal *THR4* marker. This may reflect replicative resolution of Holliday structures according to the two-strand model of Esposito⁴⁵, or may be a consequence of double-strand breaks found at *MAT*.

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Lack of association between intrachromosomal gene conversion and reciprocal exchange

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The association of reciprocal exchange with intrachromosomal gene conversion has been examined using an inverted repeat at the HIS3 locus of Saccharomyces cerevisiae. Intrachromosomal gene conversions between the duplicated inverted sequences are frequent. In contrast to gene conversion events between homologous chromosomes, intrachromosomal gene conversion is not associated with reciprocal exchange in flanking sequences. This observation has important consequences for the role of gene conversion in the maintenance of multigene families.

THE genetic interactions that occur between direct gene duplications have been studied in both the mitotic¹ and meiotic² stages of the life cycle of the yeast *Saccharomyces cerevisiae*. These studies have shown that most of the interactions are gene conversion events. Genetically this is a mechanism which produces sequence homogeneity, yielding two genes of identical genotype (and presumably identical sequence) where initially the genes were non-identical. Gene conversion events are of importance in evolutionary considerations of the behaviour of tandemly repeated genes in the germ line of higher eukaryotes^{3–8}.

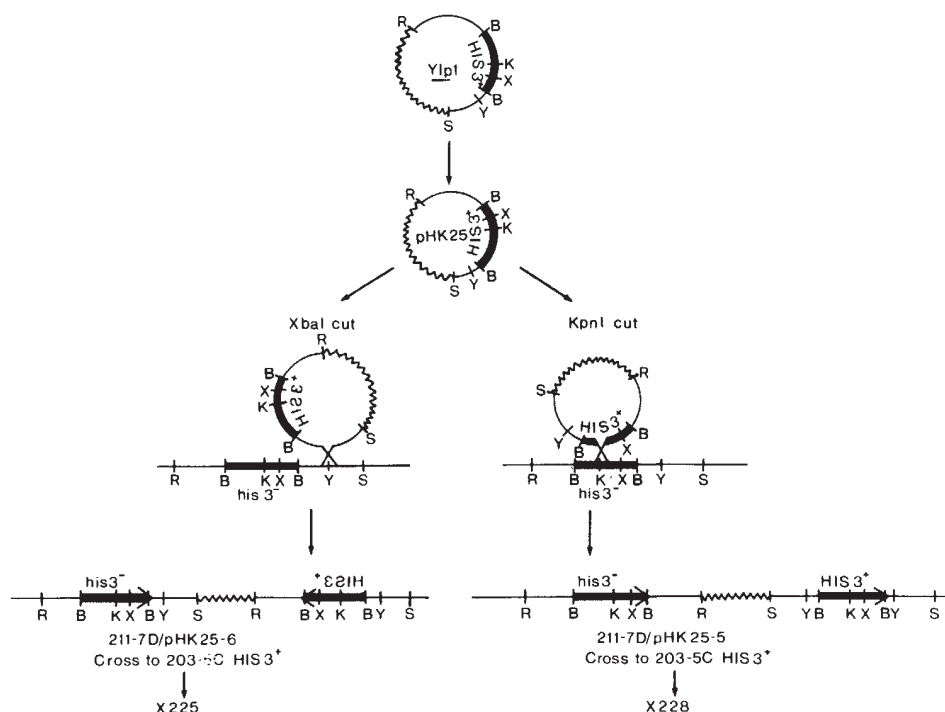
In the fungi, approximately 50% of meiotic gene conversion events are associated with outside marker exchange^{9–12}. Once the observed values are corrected to take into account incidental exchanges in the regions examined¹³, this figure falls to 35%, with the values for conversion-associated exchange frequency ranging over 0.18–0.66 (refs 10, 14–16). These studies describe

interchromosomal gene conversion events, occurring between homologues.

If intrachromosomal gene conversion were also associated with reciprocal exchange, a deletion of the sequences between the repeats would occur whenever the repeats were in the same orientation. Inversion of the sequence between the repeats would occur when the repeats were inverted. Previous experiments have suggested that reciprocal exchanges are not associated with intrachromosomal gene conversion events between direct duplicated genes^{1,2}. However, due to the particular constructions used, conversion events associated with reciprocal recombination of flanking markers could not be distinguished from reciprocal cross-overs. Both types of events will result in the loss of one copy of the duplicated gene.

Here we examine genetic interactions between an inverted duplication of the *HIS3* gene in *Saccharomyces cerevisiae*. No

Fig. 1 Construction of an inverted duplication of the *HIS3* gene. YIp1 carrying the yeast *HIS3*⁺ gene¹⁸ was cleaved with *Bam*HI and religated to give pHK25. This plasmid is identical to YIp1 with the exception that the *Bam*HI fragment is inserted in the opposite orientation. pHK25 was digested with *Xba*I or *Kpn*I and used to transform a *his3*⁻ strain to *HIS3*⁺. Using the *Xba*I-cut pHK25 plasmid, the transformant 211-7D/pHK25-6 was recovered with the *HIS3* genes in inverted orientation. Using the *Kpn*I-cut pHK25 plasmid, the transformant 211-7D/pHK25-5 was recovered. This strain carries the *HIS3* genes as a direct repeat. The restriction maps are not drawn to scale. The restriction enzyme sites are: R, *Eco*RI; S, *Sal*I; Y, *Xba*I; B, *Bam*HI; X, *Xho*I; K, *Kpn*I. Jagged line, pBR322 sequences; thin line, yeast sequences; thick line, 1.7-kb *Bam*HI fragment containing the yeast *HIS3* gene. This is the sequence that is inverted in pHK25. Small scale plasmid DNA preparations from *Escherichia coli* were prepared as described previously³². Large scale preparations of plasmid DNA were prepared according to the alkaline lysis method³³ and isolated from caesium chloride gradients containing ethidium bromide³⁴. Plasmid DNA fragments were isolated by electroelution. Transformation of *E. coli* was performed³⁵ and ampicillin-resistant transformants were selected on L broth supplemented with 100 µg ml⁻¹ ampicillin. Yeast transformations were performed³⁶ using linear plasmids²⁰.



DNA sequences are lost during reciprocal exchanges between inverted repeats. Instead the interstitial sequence between the repeats is inverted, making it possible to determine whether or not intrachromosomal gene conversion events are associated with recombination of flanking sequences.

Construction of the inverted repeat

An inverted duplication of the *HIS3* gene at the *HIS3* locus was constructed as shown in Fig. 1. The strategy was to form a plasmid carrying a large insertion of sequences from the *HIS3* region of yeast. Inversion of an internal segment containing the *HIS3* genetic information and subsequent introduction of this plasmid into the yeast chromosomal *HIS3* locus by transformation¹⁷ results in a duplication. The vector YIp1 containing a 6.1 kilobase (kb) insert from the *HIS3*⁺ gene of yeast, replacing the *Eco*RI-*Sal*I fragment of pBR322 (ref. 18), was used as the starting plasmid. The *HIS3*⁺ gene is contained on a 1.7-kb *Bam*HI fragment of this insert¹⁹. Following *Bam*HI digestion of YIp1 and ligation, the vector pHK25 was recovered. This vector now carries the 1.7-kb *Bam*HI fragment in the opposite orientation. pHK25 was then treated with *Xba*I or *Kpn*I, each of which makes only one cut in the yeast DNA. The digested plasmid DNA was used to transform strain HK211-7D (α *ade6 leu2-3, 2-112 his3-11, 3-15*) to histidine prototrophy. *Xba*I does not cut in the 1.7-kb *Bam*HI fragment while *Kpn*I does. Since the ends of molecules are recombinogenic in yeast²⁰, transformation with each of these linear plasmids should give different arrangements of the *HIS3* genes and flanking sequences. Transformants recovered after introduction of pHK25 DNA cut with *Xba*I should carry an inverted duplication of the *Bam*HI fragment but the flanking yeast sequences of pHK25 still present should be in the same orientation. Transformation with pHK25 cut with *Kpn*I should generate a direct duplication of the *Bam*HI fragment but the flanking yeast sequences should be arranged as inverted duplications.

Both these constructions gave *HIS3*⁺ transformants. To ensure that the *HIS3* genes were carried in the appropriate orientations and that only one copy of the plasmid pHK25 had been inserted, nuclear DNA from the yeast transformants was analysed. The results show that the appropriate genomic frag-

ments are altered in the transformants (Fig. 2; lanes 1, 3.). Both *Xba*I and *Xho*I cut once in pHK25 (Fig. 1). If the transformants contained more than one copy of this plasmid inserted at the *HIS3* locus, a band the size of linear pHK25 would be observed. The fact that no band of this size was detected shows that only one copy of the plasmid had been inserted. The weakly hybridizing bands in Fig. 2 are due to incomplete digestion of the yeast DNA.

Meiotic stability

One transformant carrying the *HIS3* gene in an inverted duplication, 211-7D/pHK25-6, and a transformant carrying the *HIS3* gene as a direct duplication, 211-7D/pHK25-5, were crossed to an untransformed *HIS3*⁺ strain HK203-5C to form the diploid strains X225 and X228 respectively. These diploids were analysed by standard meiotic tetrad analysis. The percentage sporulation was ~70% and the spore viability 75%.

A total of 299 tetrads giving four viable spores from X225 and 313 tetrads with complete viability from X228 were analysed. Since both homologues of chromosome XV carry a functional *HIS3* gene, all four spores of each tetrad should exhibit histidine prototrophy, giving 4⁺:0⁻ segregation. With the exception of 10 tetrads from X225 and 9 tetrads from X228, all tetrads gave this segregation.

Analysis of aberrant tetrads

All aberrant tetrads gave a segregation pattern of 3⁺:1⁻ for histidine requirement. The genetic and physical analyses described below allowed the separation of these tetrads into four classes. The genotypes of the His⁻ spores were determined by standard allelism tests. These experiments showed that all of the His⁻ spores contained both of the *his3* alleles of the parental strain HK211-7D. Such His⁻ spore segregants could easily arise from a reversal of the integration event, resulting in the loss of plasmid sequences. However, colony hybridization with ³²P-labelled pBR322 sequences showed that in all cases except one, two spores of each tetrad contained plasmid sequences. Thus, excision of the *HIS3*⁺ gene cannot account for the His⁻ phenotype.

The number of *HIS3* genes present in each strain was

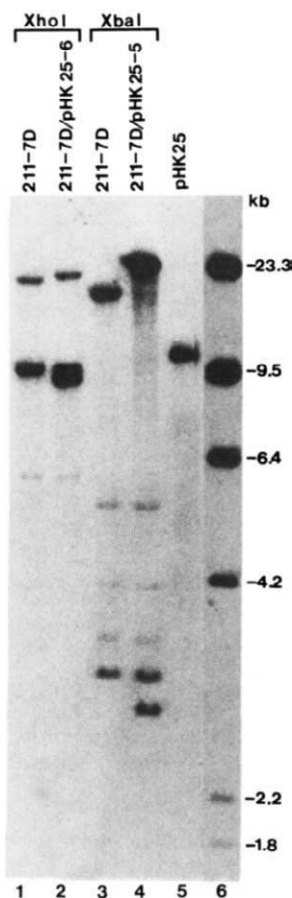


Fig. 2 Identification of the pHK25 transformants. Nuclear DNA was digested with *Xba*I or *Xho*I and separated on a 0.7% horizontal agarose gel slab by electrophoresis at 35 V cm^{-1} for 40 h. Following transfer to nitrocellulose³⁷ the filter was hybridized with ^{32}P -labelled³⁸ YIpl DNA. Yeast nuclear DNA was prepared as previously described³⁶. Restriction enzyme digestions and ligation reactions were performed according to the conditions recommended by the supplier. Approximately $5 \mu\text{g}$ yeast nuclear DNA was used in each restriction enzyme digestion. Hybridization reactions were performed at 65°C in 6X SSC, 2% SDS, 1X Denhardt's solution³⁹. Filters were exposed to Kodak XAR-5 film at -70°C with intensifying screens⁴⁰. Lanes 1, 3, untransformed strain HK211-7D; lane 2, HK211-7D/pHK25-6; lane 4, HK211-7D/pHK25-5; lane 5, pHK25. Lanes 1, 2 and 5 were digested with *Xho*I; lanes 3 and 4 were digested with *Xba*I. Lane 6 contains λ DNA digested with *Hind*III for size markers.

determined. Nuclear DNAs isolated from segregants of X225 were digested with *Xho*I while those from X228 were digested with *Xba*I. All spores lacking plasmid sequences gave hybridization patterns identical to the untransformed strain, while spores containing plasmid sequences gave patterns identical to the parental transformant, with the exception of one spore that contained an additional band that co-migrated with linear plasmid pHK25.

The genotypes of the *HIS3* genes in strains that contain two copies of the *HIS3* gene and are prototrophic for histidine were determined by UV mutability tests². These analyses allow the tetrads to be grouped into four classes, (Fig. 3). The number of tetrads of each class from X225 and X228 is indicated.

Class I tetrads result from an intrachromosomal gene conversion event. This is a transfer of genetic information from one gene, in this case the *his3-11, 3-15* mutant gene, to the wild-type copy of the gene on the same chromosome. The resulting spore is *His*⁻, carries the same alleles as the original untransformed strain HK211-7D and contains pBR322 sequences and two copies of the *HIS3* gene. This class was recovered at a frequency of 2% from X225 and 0.7% from X228.

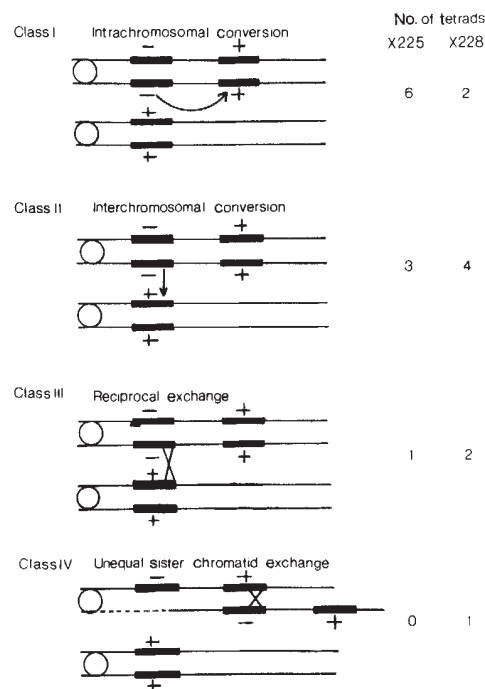


Fig. 3 Schematic explanations of the interactions giving rise to the different classes of aberrant tetrads. Open circles, centromeres; dark lines, *HIS3* sequences; +, wild-type genotype; -, mutant. The order of genes with respect to the centromere is drawn according to Sugawara and Szostak⁴¹. The *his 3-11, 3-15* mutations are at the 5' end of the gene which is proximal to the centromere. The arrows in the class I and II entries indicate gene conversion events, with a change of the + gene next to the arrowhead to a - gene, while the - gene remains unchanged. The columns on the right indicate the number of tetrads in each class recovered from diploids X225 and X228.

Class II tetrads result from gene conversion between homologues. In these tetrads the *His*⁻ spore contains the original *his3* alleles, but in contrast to class I tetrads, lacks pBR322 sequences and has only one copy of the *HIS3* gene. Spores from those tetrads that contain pBR322 sequences are *HIS*⁺, but carry one mutant *his3* gene as determined by the mutability tests. This class was recovered at a frequency of 1–1.3%. This is approximately the same frequency as the class I tetrads.

Class III tetrads result from reciprocal exchange between homologues. As in the case of the class II tetrads, the *His*⁻ spore lacks plasmid sequences. However, one of the *HIS*⁺ spores should yield few *His*⁻ segregants, and none carrying the *his3-11* or *his3-15* alleles following UV treatment, as this spore contains two wild-type copies of the *HIS3* gene.

The single tetrad comprising class IV was recovered from X228 and can be explained by an unequal sister chromatid exchange. The following evidence supports this contention. First, only one *HIS*⁺ spore contains plasmid sequences while the *His*⁻ spore lacks plasmid sequences. Second, it was extremely difficult to recover any *His*⁻ segregants from the *HIS*⁺ spore containing plasmid sequences. Third, Southern analysis gave a pattern different from those observed in the other tetrads. Three lacked plasmid sequences while the spore with plasmid sequences had three copies of the *HIS3* gene, with one band the size of the linear pHK25 plasmid.

Assay for reciprocal exchange

Reciprocal cross-overs associated with gene conversion events between inverted repeats can be easily detected because the cross-over will result in an inversion of the interstitial sequence. The outcome of pairing along the 1.7-kb *Bam*HI fragment duplicated in strain 211-7D/pHK25-6 as an inverted duplication and crossing-over in this region is shown in Fig. 4a. With the exception of the right-hand *HIS3* gene, the duplication has the

same order and spacing of restriction sites as strain 211-7D/pHK25-5 (compare Figs 1 and 4a). Reciprocal exchange can be detected by *Xba*I digestion and Southern blot analysis of nuclear DNAs from the aberrant tetrads of X225. If no exchange has occurred within the *Bam*HI fragment, then digestion with *Xba*I and hybridization with ³²P-labelled YIpl sequences should give two bands of hybridization in spores that have one copy of the *HIS3* gene, while spores containing two copies of the *HIS3* genes should contain an additional band of linear pHK25 plasmid size. If exchange has occurred between the inverted repeats, then a different pattern of hybridization will be observed which will be identical to that of 211-7D/pHK25-5 (Fig. 2). This analysis was performed on all the aberrant tetrads of X225. Examples of three analyses, from tetrads 8, 55 and 89, are shown in Fig. 5. There is 2:2 segregation for each spectrum of bands of hybridization. In addition, no deviations from the patterns of the parental strains HK211-7D/pHK25-6 and HK203-5C were seen. This pattern was the same for all the tetrads. Tetrads from X228 showed no rearrangements when compared with the parental strains HK211-7D/pHK25-5 and HK203-5C following *Xho*I digestion and Southern analysis except in the case of one tetrad. This will be described in more detail later.

In the six cases of intrachromosomal gene conversion in the inverted *HIS3* duplication of X225, no reciprocal exchange was observed. Because the sample size was small, additional examples of intrachromosomal gene conversion were recovered by random spore analysis of X225. 109 His⁻ spores were recovered from ~10,000 single spore colonies. All of these His⁻ spores contained the *his3-11*, *3-15* alleles; 36 contained pBR322 sequences while 73 did not. Nuclear DNA was prepared from these 36 strains and analysed, as described above, for reciprocal exchange events in the 1.7-kb *Bam*HI fragment. Again, no reciprocal cross-overs were observed in this conversion class.

Viability of cross-over products

Struhl and Davis²¹ have shown by Northern blot analysis that three RNA species are transcribed from the region around *HIS3* that is inserted in YIpl. One of these transcripts is in high abundance. Although all the duplication constructions and possible rearrangements by reciprocal exchange events should contain at least one intact DNA sequence corresponding to each transcript, it is possible that some of these are lethal arrangements, owing to position effects. As the analysis to detect reciprocal exchange associated with intrachromosomal gene conversion was done with haploid strains (spore segregants), it was necessary to exclude lethality as a trivial explanation of the failure to detect any of these events.

During the analysis of tetrads from X228 to detect rearrangements, one spore segregant was found with a novel pattern of hybridization. This is the result of an intrachromosomal gene conversion and a reciprocal cross-over unrelated to the conversion event. Spore 228-324A is His⁻. Fig. 6a shows the results following *Xba*I or *Xho*I digestion of DNA from this tetrad. Although the arrangement of sequences at the *HIS3* locus in strain 228-324A does not seem to differ from the other spore with the *HIS3* duplication, 228-324D, when treated with *Xba*I or *Bam*HI (data not shown), different results are seen when *Xho*I is used. The differences are indicated by the arrowheads. The change in band migrations can be explained by the reciprocal exchange shown in Fig. 4b. *Xba*I digestion and Southern blot analysis should give bands identical to the parental strain HK211-7D/pHK25-5, which contains a direct repeat of the 1.7-kb *Bam*HI fragment, whereas *Xho*I digestion should give a pattern identical to that obtained from *Xho*I digestion of the parental strain HK211-7D/pHK25-6, which contains the inverted repeat of the 1.7-kb *Bam*HI fragment (Fig. 6b).

Two points should be noted here. First, strain 228-324A is a haploid strain by all genetic criteria. Second, the arrangement of the duplicated sequences at the *HIS3* locus shown in Fig. 4b is identical to that shown in Fig. 4a resulting from reciprocal

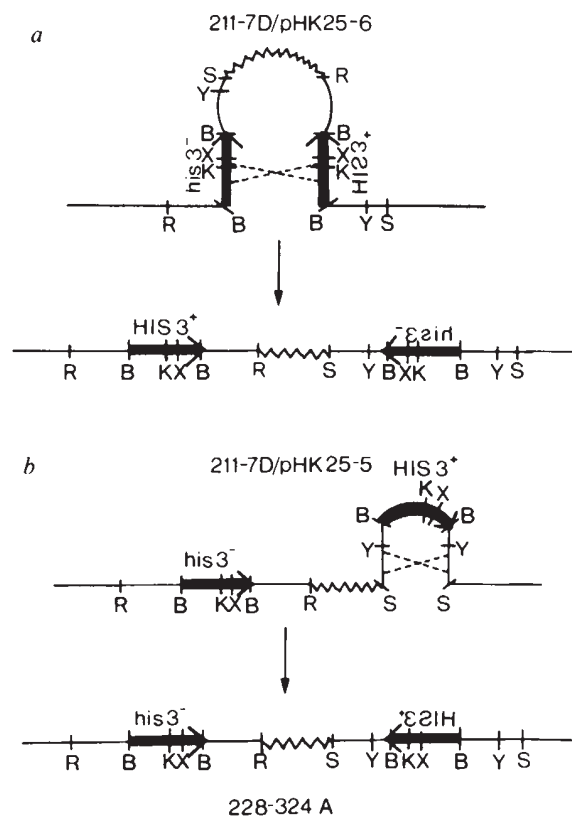


Fig. 4 Predicted outcome of exchange between the inverted repeats. *a*, The chromosome of 211-7D/pHK25-6 is shown pairing along the inverted 1.7 kb *Bam*HI sequences. Reciprocal exchange between these two sequences will give the structure shown. Gene conversions are not shown. *b*, The chromosome of 211-7D/pHK25-5 containing the *HIS3* genes in direct orientation is shown pairing along the inverted *Sal*I-*Bam*HI sequences flanking the *his3*⁻ gene. Reciprocal exchange between these sequences gives the DNA sequence organization of strain 228-324A. The gene conversion event is not shown. The restriction enzyme maps are not drawn to scale. Thin lines, yeast sequences; thick line, yeast 1.7 kb *Bam*HI sequences containing the yeast *HIS3* gene; jagged line, pBR322 sequences. The restriction sites are: R, *Eco*RI; B, *Bam*HI; K, *Kpn*I; X, *Xho*I; Y, *Xba*I; S, *Sal*I.

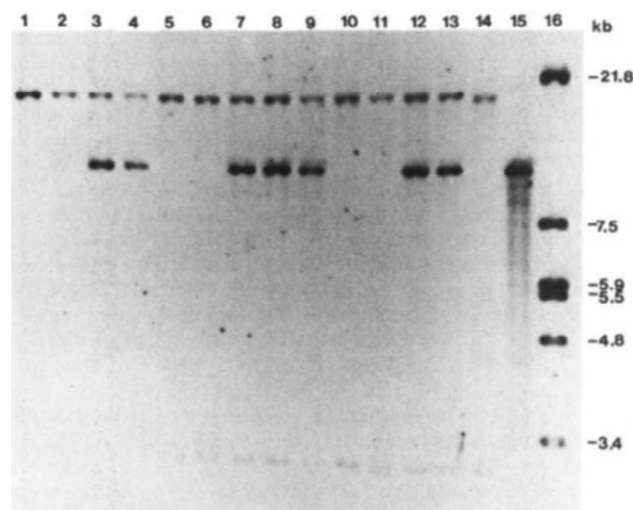


Fig. 5 Southern blot analysis of tetrads from X225. Lanes 1-4, tetrad 8 spores A-D; lanes 5-8, tetrad 55 spores A-D; lanes 9-12, tetrad 89 spores A-D; lane 13, HK211-7D/pHK25-6; lane 14, HK203-5C; lane 15, pHK25; lane 16, λ DNA digested with *Eco*RI for size markers.

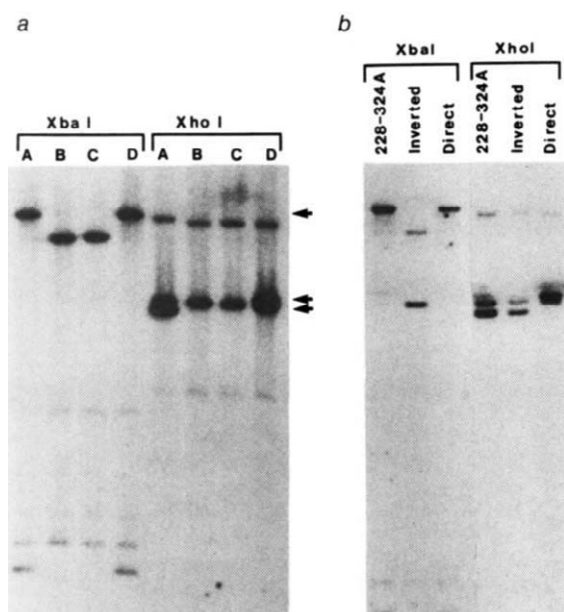


Fig. 6 Rearrangement of the *HIS3* duplication in 228-324A. *a*, Spores A–D from tetrad 324 of X228 digested with either *Xho*I or *Xba*I. The arrowheads indicate the band changes between spores A and D following *Xho*I digestion. *b*, 228-324A was electrophoresed with the inverted repeat of the *HIS3* gene, strain HK211-7D/pHK25-6, and the direct repeat of the *HIS3* gene, strain HK211-7D/pHK25-5, following digestion with *Xba*I or *Xho*I.

exchange in the 1.7-kb *Bam*HI fragment in the inverted duplication. Thus, failure to recover this among the intrachromosomal gene conversion events cannot be due to lethality.

Discussion

Meiotic intrachromosomal gene conversion and associated outside exchange have been examined using inverted repeats at the *HIS3* locus. Using unselected tetrads and random spores, 42 examples of intrachromosomal gene conversion between inverted repeats of the *HIS3* gene have been analysed. The frequency of intrachromosomal conversion (0.7–2%) is the same as that observed at the *LEU2* locus². It is also the same as the frequency of interchromosomal gene conversion at the *HIS3* locus observed in X225 and X228 (this study) and is similar to values observed at other loci in yeast¹¹. In contrast to interchromosomal gene conversion, no outside exchange is associated with the intrachromosomal gene conversion events studied here, in spite of the fact that such an event does not lead to cell inviability. This is consistent with an earlier study of intrachromosomal gene conversion events at the *LEU2* locus² in which no reciprocal exchanges were found to accompany conversion. Furthermore, in over 500 meiotic interchromosomal conversion events associated with the serine tRNA genes located on non-homologous chromosomes, none resulted in translocations or other lethal rearrangements (P. Munz, personal communication).

During mitotic recombination between homologous genes on nonhomologous chromosomes, the frequency of gene conversion events is 10-fold higher than the frequency of reciprocal exchanges²². In addition, intrachromosomal gene conversion during mating type switching in yeast is not normally associated with reciprocal exchange^{23–27}. The *MAT* locus is the recipient for the transfer of information from one of the two cassette loci located on the end of each arm of the chromosome carrying the *MAT* locus. However, when mating type switching is forced to occur between homologues, outside exchange is observed²⁸ sug-

gesting that gene conversion at this locus is not inherently constrained from being associated with reciprocal exchange.

It could be argued that most of the intrachromosomal gene conversion events occur between sister chromatids and are not in fact intrachromatid. If these interactions were associated with outside exchange, then in the case of the inverted repeats the final meiotic produce would be a dicentric, lacking sequences distal to the *HIS3* locus. This would be lethal and hence not recovered in this study, as only tetrads with four viable spores were analysed. Although sister chromatid interactions cannot be ruled out, unequal sister chromatid exchanges between direct repeats of 2–4 kb are rare (this study and ref. 2). Moreover, the unequal sister chromatid exchanges observed in these cases are not associated with gene conversions.

The absence of reciprocal exchange associated with intrachromosomal gene conversion demonstrated here is particularly attractive with regard to the events involved in the maintenance of sequence homogeneity of multigene families. If reciprocal exchange were associated with half the intrachromosomal conversion or correction events, then deletions or inversions depending on the relative orientation of the two genes would result. Such chromosomal rearrangements might be lethal, making the gene correction mechanism rather inefficient. Indeed, the examples of gene conversion between members of a multigene family on one chromosome which have been proposed show no evidence of other exchange events associated with the conversion.

The association of outside exchange with gene conversion is thought to be due to the mechanism of resolution of the recombination intermediate. Isomerization of a crossed-strand structure of a Holliday junction²⁹ followed by nicking and ligation would lead to recombinant outside markers³⁰. The ratio of recombinant to non-recombinant outcomes should depend upon the rate of isomerization and equilibrium of the two forms. The observation that exchange is associated with conversion 50% of the time suggests that isomerization occurs rapidly and that the two forms are in equilibrium, although it has been argued that isomerization is not readily effected³¹. Pairing between short regions of homology may not provide enough stability to permit isomerization. Alternatively, intrachromosomal pairings may not present the proper configuration to the isomerization machinery, with the result that intrachromosomal gene conversions are always nonrecombinant for flanking sequences.

In conclusion, meiotic intrachromosomal gene conversion in yeast is frequent. However, it has never been observed in association with reciprocal exchange. This is in contrast to gene conversion between homologues. The lack of association of reciprocal exchange with intrachromosomal gene conversion could explain why multigene families are able to exchange information frequently by gene conversion without associated chromosome rearrangements.

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LETTERS TO NATURE

Resonance locking between Jupiter and Uranus

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Kinoshita and Nakai¹ have recently performed the longest numerical integration of the outer bodies of the Solar System ever attempted. The positions and velocities of Jupiter, Saturn, Uranus, Neptune and Pluto for the 5 Myr that they computed extended the integration time by a factor 5 with respect to previous ephemerides computed by Cohen *et al.*². Although this is only one-thousandth of the present age of the Solar System, our analysis of the data using new methods^{3–5}, shows surprising dynamical features. Stability is monitored by separating the system into three-body subsystems. It is found that the outer Solar System is made up of two main subsystems, Sun–Jupiter–Saturn and Sun–Uranus–Neptune, exchanging angular momentum over a period of 1.1 Myr. The dynamical mechanism responsible for locking the two subsystems together is a libration of the angle between Jupiter's and Uranus' perihelion around 180° with the same period of 1.1 Myr. No dynamical locking over such a long period of time has been found in the Solar System, although similar mechanisms have been predicted.

We have examined one magnetic tape from Kinoshita and Nakai containing the output of the 5 Myr integration, that is, planetary ephemerides once every 2,000 days. They used updated values of planetary masses⁶; the total mass of the inner planets, which perturb only slightly the outer ones, was added to the mass of the Sun. We analysed the data in order to investigate the stability of the outer Solar System over 5 Myr. In the past 15 yr, two major breakthroughs should help attempts to prove the stability of the Solar System for a time comparable to its age, of ~4,500 Myr. (1) For an isolated three-body system, using no approximation (general three-body problem), a stability criterion has been assessed which is valid for all time and depends on the value of one single scalar parameter of the system, $z = c^2 h$ (total angular momentum squared times total energy)^{3,7–10}. (2) It has been pointed out that N -body systems existing in nature (with N of the order of 10) mostly show an hierarchical arrangement, and that this does not happen by chance^{4,11}. In the Solar System, the planetary orbits do not cross each other (except for Pluto and Neptune); most of the observed three and four-body stellar systems are a close pair with a distant companion or two close pairs at a large distance. Also the six-body stellar system, Castor, shows an hierarchical structure. That is, dynamical systems tend to be hierarchically arranged in such a way that mutual gravitational perturbations are minimized. Systems that were not hierarchical simply did not survive long enough to be observed.

This suggests a new way of investigating the hierarchical stability of the Solar System (that is, the system is hierarchically

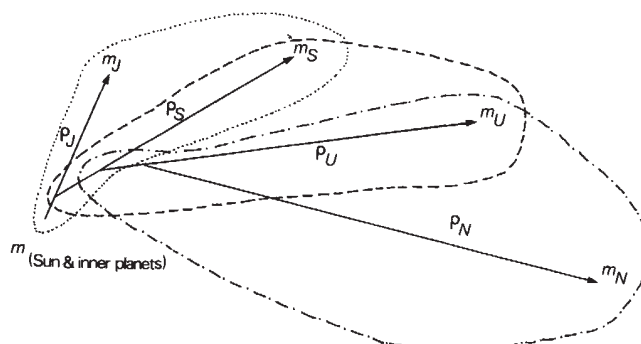


Fig. 1 Three-body subsystems of the outer Solar System. The 'tail' of each position vector p_J , p_S , p_U , p_N is in the centre of mass of the Sun plus all the preceding planets. Not to scale.

stable as long as no orbit crossing occurs). First, it is decomposed into three-body subsystems. In the case of Sun–Jupiter–Saturn–Uranus–Neptune, we have three three-body subsystems (Fig. 1): Sun–Jupiter–Saturn, Sun–Saturn–Uranus and Sun–Uranus–Neptune (the Sun always includes the mass of all the preceding bodies). Second, by applying the z stability criterion to each subsystem at present, we find that it is fulfilled in all three cases. This means that if they were three isolated three-body systems, Jupiter would never cross the orbit of Saturn, Saturn would never cross the orbit of Uranus, Uranus would never cross the orbit of Neptune and each system would always remain hierarchically stable. But, because they are not isolated, and do perturb each other, the three z stability parameters change with time and could, in principle, reach the corresponding critical values above which hierarchical stability is no longer guaranteed. As long as all the stability parameters remain below the critical values, the whole system is guaranteed to be hierarchically stable.

Therefore, the problem arises of monitoring the z stability parameters as functions of time. This is possible for the 5 Myr span of Kinoshita and Nakai's numerical integrations. We have computed energies, angular momenta and z parameters of three-body subsystems at intervals of 2×10^5 days (~547 yr); then they have been averaged 10 by 10. The interval 5 Myr is very short compared with the age of the Solar System. Moreover, because the Solar System is an hierarchical dynamical system, one would expect that, apart from short period variations related to near-resonances in mean motions between major planets², the z parameters would be constant over 5 Myr. What we find is, at first sight, surprising (Fig. 2a, b, c). The three z parameters, although always lower than the corresponding critical value (hierarchical stability of the whole system is guaranteed for 5 Myr), show a long-term oscillation with a period of ~1.1 Myr. In the Sun–Saturn–Uranus case (Fig. 2c) the dominant oscillation

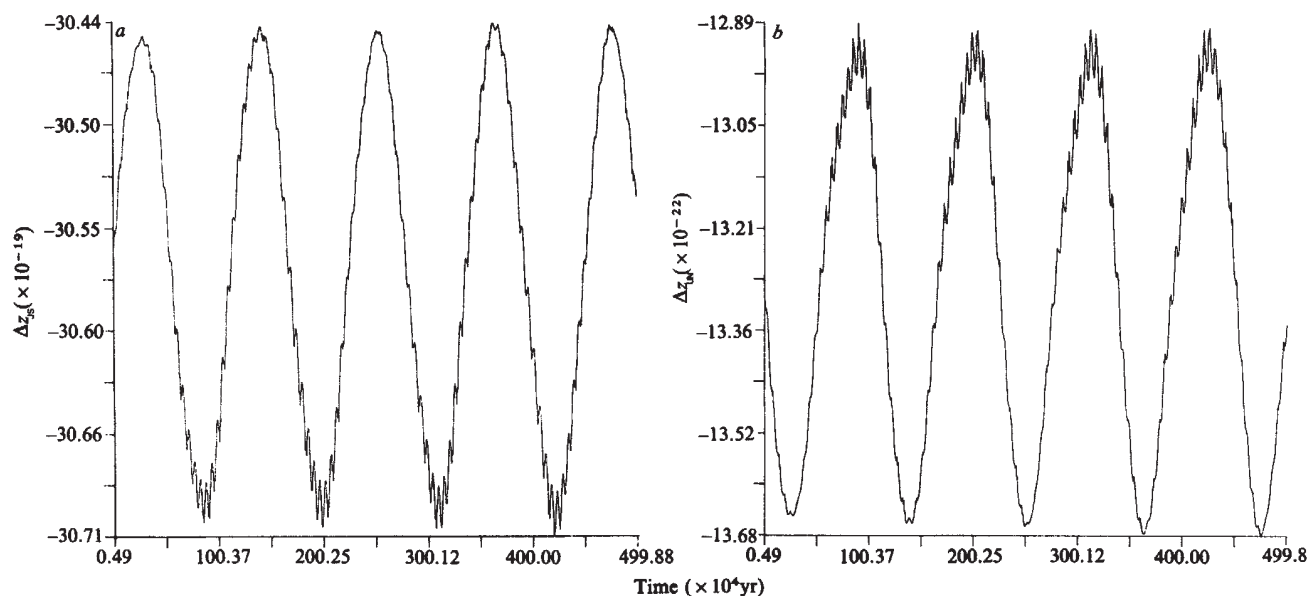


Fig. 2 The difference between the actual and the critical values of the z stability parameters as functions of time for 5 Myr: the more negative the value, the more stable is the three-body subsystem. *a*, Sun-Jupiter-Saturn; *b*, Sun-Uranus-Neptune; *c*, Sun-Saturn-Uranus. *a*, *b*, Oscillations with the same period of ~ 1.1 Myr but opposite in phase; the more stable is Sun-Jupiter-Saturn, the less stable is Sun-Uranus-Neptune. In *c*, the dominant oscillation has a period of 54,000 yr, although the 1.1 Myr period is also shown.

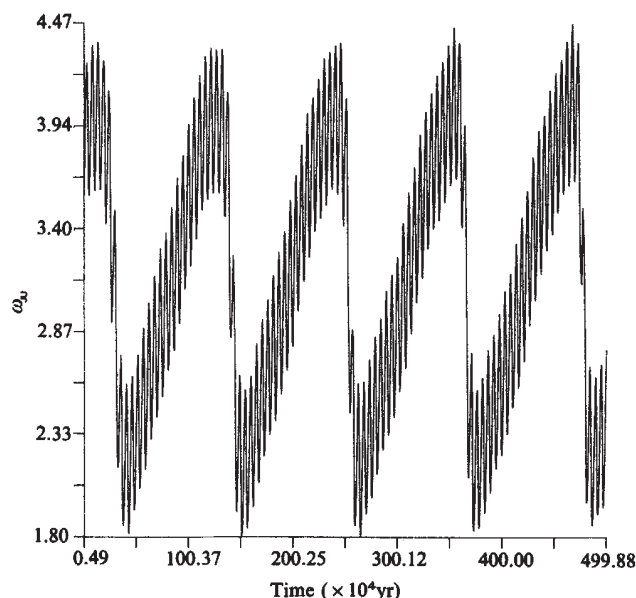


Fig. 3 The angle ω_{JU} between the jovian and uranian pericentres (in rad) as a function of time for 5 Myr. The libration around π with a period of 1.1 Myr is apparent.

tion contains the 54,000-yr period of Jupiter's perturbation on Saturn², modulated over 1.1 Myr. As expected^{4,12}, its relative amplitude is the largest that we find. Even more striking is that for Sun-Jupiter-Saturn and Sun-Uranus-Neptune subsystems, the oscillations are opposite in phase, as though the whole system were made of these two main subsystems exchanging 'stability' over 1.1 Myr.

What is the dynamical mechanism responsible for this long-period perturbation in the stability parameters? A clue is provided by the fact that the angular momenta show the same pattern and the same exchange between subsystems as do the stability parameters, whereas the energies do not. This means that the resonance locking mechanism must involve the pericentres. We actually find the result shown in Fig. 3: the angle ω_{24} between the jovian and uranian pericentres does not circulate; it librates around 180° within about $\pm 70^\circ$ in the same period of 1.1 Myr. (For a more detailed discussion of these results see ref. 12.)

Pluto was not included in the analysis because it crosses the orbit of Neptune and, moreover, its mass is too small for the z criterion to be applicable^{3,5}. Its motion is discussed by Kinoshita and Nakai¹.

The hierarchical stability of the whole Solar System remains open to question. Because there is no suitably long numerical integration available, we have tackled the problem using analytical methods⁴. We find a lower limit for the lifetime of the

Sun-Mercury-Venus-Jupiter system of 110 Myr. In the whole Solar System, the main problem is the strong mutual perturbation between Jupiter and Saturn which makes our methods ineffective over the long time intervals of interest to us.

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Predictions for Uranus from a radiometric Bode's law

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The magnetospheres of three planets, Earth, Jupiter and Saturn, are sources of intense, nonthermal radio bursts. Recent studies¹⁻³ have shown that the emissions from these sources undergo pronounced long-term intensity fluctuations that are caused by the solar wind interaction with the magnetosphere of each planet. Determinations by spacecraft of the low-frequency radio spectra and radiation beam geometry now permit a reliable assessment of the overall efficiency of the solar wind in stimulating these emissions. We find that earlier estimates⁴ of how magnetospheric radio output scales with the solar wind energy input must be greatly revised, with the result that, while the efficiency is much lower than previously thought, it is remarkably uniform from planet to planet. This result has prompted our formulation a 'radiometric Bode's law' from which a planet's magnetic moment can be estimated from its radio emission output. (This terminology is by analogy with the 'magnetic Bode's law', sometimes called Blackett's law⁵.) Applying the radiometric scaling law to Uranus, we estimate the low-frequency radio power likely to be measured by the Voyager 2 spacecraft as it approaches this planet. We also show how measurements of the Uranus radio flux by Voyager 2, which will probably be made in early to mid-1985, can be used to estimate the planetary magnetic moment and solar wind stand-off distance before the *in situ* measurements.

We define the efficiency ε as the ratio of the median power radiated in magnetospheric emissions, P_r , to the incident solar wind power, P_i , in average solar wind conditions. The median radiated power is typical of that observed in average solar wind conditions. The starting point for the calculation of P_r is integration over frequency of the median flux density spectra shown in Fig. 1. The total radiated power (W) is then the resulting integral multiplied by the area into which the emission is beamed. Thus, in the case of Saturn and the Earth, the integral is over the entire known flux spectrum since the auroral (terrestrial) kilometric radiation (AKR) and Saturn kilometric radiation (SKR) are both solar wind controlled. For Jupiter, only the hectometre-wavelength emission (HOM), which is known to be solar wind controlled², contributes significantly to the total solar wind driven output. Therefore, only that portion of the spectrum, from ~200 kHz to ~3 MHz (undotted portion of the jovian spectrum in Fig. 1), is relevant in this analysis. To compute the area into which the emission is radiated, the beam geometry of each source must be known. Saturn's northern hemisphere

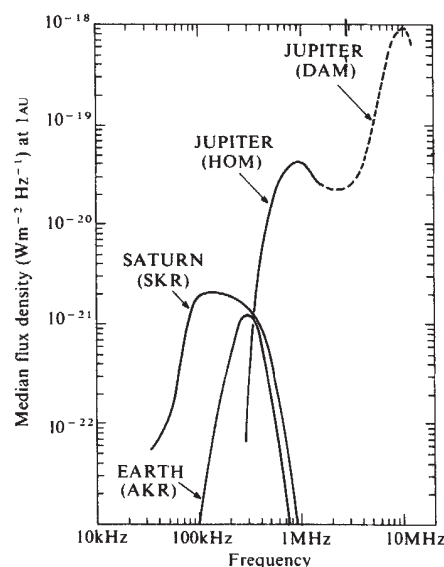


Fig. 1 Low-frequency median flux density spectra for the three known radio planets, Earth⁹, Jupiter¹⁰ and Saturn⁶, are shown with the solar wind driven components indicated by solid lines. Flux densities are normalized to what an observer would measure at a distance of 1 AU from each planet. For clarity, the kilometre-wavelength components of Jupiter's emission are not shown.

emission has been shown⁶ to radiate into a filled 2π steradian (sr) conical beam and Earth's into a more narrow 3.5 sr beam⁷. In contrast, while Jupiter's highest frequency burst emission, the decametre-wavelength emission (DAM), is very narrowly beamed, the HOM is more moderately beamed into a wide, relatively thin, $\sim \pi/2$ sr surface⁸.

Total radiated powers of the terrestrial⁹, jovian¹⁰ and saturnian⁶ radio sources have been estimated in previous studies. However, only the value for AKR derived by Kaiser and Alexander⁹ applies without modification in the present analysis because they used the median flux radiated into the actual emission beam. For Jupiter, we have modified an earlier 3×10^{10} W isotropic (4π sr) calculation¹¹ to reflect the modelled ($\pi/2$ sr) HOM radiation beam cited above. Similarly, we have halved the value used previously for Saturn's SKR⁶, which was for an isotropic (4π sr), not a 2π sr, radiator. Thus, we have been careful throughout to use the measured or inferred beam geometry of each source and to include only that portion of each planet's median burst spectrum that is known to be solar wind driven. Finally, the emitted power was doubled in the case of both Saturn and the Earth to account for radiation from the opposite (southern) hemisphere. It is not known if the jovian HOM has a southern hemisphere component, and since modelling efforts⁸ have suggested a single source we have not doubled the measured power.

The solar wind input P_i at each planet was computed from

$$P_i = \pi m_p \rho_0 V^3 (l_0/d)^2 \quad (1)$$

which is a measure of the power (W) incident on the cross-sectional area of the magnetosphere due to the bulk motion of the solar wind plasma. Here, m_p is the proton mass, ρ_0 is the solar wind number density at the Earth, V is the average solar wind bulk speed, l_0 is the solar wind-magnetosphere stand-off (Chapman-Ferraro) distance, and d is the planet-Sun distance expressed in AU ($1 \text{ AU} = 1.5 \times 10^{11} \text{ m}$). We have taken values at each planet that apply in average solar wind conditions, that is, $\rho_0 = (7 \times 10^6 \text{ m}^{-3})$, $V = 4 \times 10^5 \text{ m s}^{-1}$, and $l_0 = 10 R_e$ ($1 R_e = 6.36 \times 10^6 \text{ m}$), $50 R_j$ ($1 R_j = 7.14 \times 10^7 \text{ m}$) and $20 R_s$ ($1 R_s = 6.03 \times 10^7 \text{ m}$), at Earth, Jupiter and Saturn, respectively. The last term, d^2 , accounts for the inverse-distance-squared decrease in the mean solar wind density beyond 1 AU.

Table 1 Efficiency comparison

Planet	P_r (W)	Beam (sr)	Spectrum used	P_i (W)	ϵ ($\times 10^{-6}$)
Jupiter	4×10^9	$\pi/2$	HOM only	1.1×10^{15}	3.6
Saturn	2×10^8	2π	Full	3.8×10^{13}	5.3
Earth	6×10^7	3.5	Full	9.6×10^{12}	6.2

Table 1 summarizes the total radiated and total input powers derived in the present study and also the spectral and beam geometry characteristics of each radio source. The resulting efficiencies ($\epsilon = P_r/P_i$) are extremely small, $\sim 0.0005\%$. These are much smaller than those previously derived⁴, primarily owing to selection in this study of only the solar wind driven emission and to better information, from spaceborne radio experiments, on the actual beaming properties and median emission spectra of each source. Although the efficiencies may be as much as an order of magnitude larger in very active solar wind conditions³, they are still extremely small when compared with the overall efficiencies of other magnetospheric phenomena, such as UV auroral output, which in the case of the Earth¹² has an efficiency nearer 0.1%. This underlines the fact that radio emission processes represent a relatively insignificant fraction of the total solar-magnetosphere energy budget.

Note also that ϵ hardly varies from planet to planet. For example, while P_r varies by over two orders of magnitude, the efficiencies are all within a factor of 1.7 of each other. This empirical result suggests that the solar wind driven components of the planetary radio sources are subject to a simple scaling relationship in which the total radiated power is directly proportional to the solar wind power incident on the planet's magnetosphere, the constant of proportionality being $\sim 5 \times 10^{-6}$.

If P_r is directly proportional to P_i through the constant of proportionality ϵ , then P_r is also directly related, through equation (1), to $(l_0/d)^2$. Using the data of Table 1, we illustrate this relationship in Fig. 2, where the radiated power P_r is plotted as a function of $(l_0/d)^2$ on the left and versus $M^{2/3}/d^{4/3}$ on the right. M , the planet's magnetic moment, is derived from the solar wind stand-off distance through

$$l_0 = (M^2/2\pi m_p \rho V^2)^{1/6} \quad (2)$$

where $\rho = \rho_0/d^2$ and which includes the effect of magnetopause surface currents¹³. The line is a least-squares fit to the data and we refer to the relationship shown in Fig. 2 as the radiometric Bode's law. With l_0 in km, d in AU and P_r in W, the fit is given by

$$(l_0/d)^2 = 6.5 P_r^{1.13} \quad (3)$$

Equation (3) permits the estimation of a planet's stand-off distance and magnetic moment based on measurement of the median power flux from the planet's solar wind driven radio emission.

Also shown in Fig. 2 is a prediction for Uranus based on an extrapolation of the radiometric Bode's law to small values. It is not known whether Uranus is a radio source^{14,15}. However, recent far UV observations^{16,17} of H Ly α emissions with the IUE spacecraft have been interpreted as due to a Uranus aurora, in which case the planet certainly possesses a magnetosphere

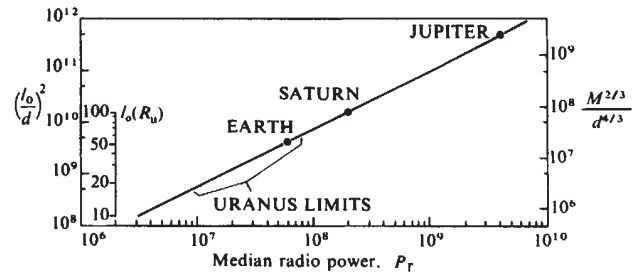


Fig. 2 The radiometric Bode's law. The total median radio power P_r (W) is plotted as a function of $(l_0/d)^2$ on the left- and versus $M^{2/3}/d^{4/3}$ on the right-hand side. Plausible range for Uranus is shown. Inset scale labelled $l_0(R_u)$ refers to solar wind stand-off distance at Uranus in units of R_u ($=23,800$ km). l_0 , d , and M are expressed here in units of km, AU and $G\text{-km}^3$, respectively.

and will probably also emit low-frequency radio emission. Table 2 summarizes the radio power levels, P_r , that we would expect from a magnetosphere having a given magnetic moment M_u . Uranus' spin axis is tilted near 90° to its orbit plane, and the Voyager spacecraft observations inbound to the planet will be nearly pole-on. Hence, only the radio emission from one hemisphere of the planet will be detectable and so the tabulated values of P_r are twice those likely to be measured initially by the Voyager radioastronomy experiment. The stand-off distance l_0 and incident solar wind power P_i resulting from each value of M_u are also shown.

Note that the range of magnetic moments shown in Table 2 is large. Figure 2 shows, however, what we consider to be a more plausible range of M_u , based on two constraints. At the low end, we do not consider it likely that M_u is $\leq 0.1 G\text{-}R_u^3$ for the following reason. If we assume a 1% conversion efficiency of solar wind power into precipitating electron flux, a smaller magnetosphere would not intercept sufficient power to drive the $\geq 10^{10}$ W emitted by the Uranian aurorae (see ref. 18). At the high end, $4.0 G\text{-}R_u^3$ represents the magnetic moment recently derived¹⁹ theoretically from external rotating dynamo considerations. In comparison, the magnetic Bode's law⁵ estimate of the Uranus field strength based on angular momentum considerations is about $1 G\text{-}R_u^3$, intermediate between these two extremes.

Reference to the inset scale, labelled $l_0(R_u)$ in Fig. 2, shows directly how the total median radio power scales with the magnetopause stand-off distance at Uranus ($R_u = 23,800$ km). For example, measurement of a Uranus radio source having a median radiated power equal to 5×10^6 W, or 10^7 W total from both northern and southern hemispheres, would require a stand-off distance of nearly $20 R_u$. This is equivalent to a $1.9 \times 10^{12} G\text{-km}^3$ ($\sim 0.14 G\text{-}R_u^3$) magnetic moment.

At present, Voyager 2 is about 6 AU from Uranus and no radio emissions have been detected by the planetary radio astronomy (PRA) instrument²⁰. This fairly conclusively rules out the detections of very strong radio bursts tentatively reported by Brown¹⁴ using the IMP-6 spacecraft, because bursts of the reported magnitude should have been observed by Voyager 2 when it was as much as 14 AU from Uranus. Assuming that the

Table 2 Uranus ratio emission and magnetic moments

M_u ($G\text{-}R_u^3$)	$l_0(R_u)$	Solar wind power (W) ($1 = 1.1 \times 10^{15}$)	P_r (W)	Comments
1×10^{-4}	<2	1×10^{-5}	$<2 \times 10^{-5}$	Minimum M_u for Uranus to stand-off solar wind.
0.01	8	2×10^{-4}	2×10^6	
0.1	18	1×10^{-3}	9×10^6	Minimum M_u to support observed auroral emission.
1.0	38	5×10^{-3}	3×10^7	M_u from magnetic Bode's law ⁵ .
4.0	61	1×10^{-2}	8×10^7	Hill <i>et al.</i> 's ¹⁹ magnetic moment.
20.0	104	4×10^{-2}	2×10^8	Minimum M_u needed to drive IMP-6 radio bursts ¹⁴ .

*Expected total power from radiometric Bode's law, for a given M_u .

radiometric Bode's law applies at Uranus, the IMP-6 bursts would have required $M_u = 20 G-R_u^3$ (see Table 2) and so the possibility of a magnetic field this large is not considered likely. Taking the plausible range of magnetosphere size described above (0.1 to 4 $G-R_u^3$), we estimate that Uranus radio emission should be easily detectable by the PRA instrument some time between December 1984 and May 1985. This will permit the planet's stand-off distance and magnetic moment to be assessed before the Voyager-Uranus encounter in January 1986.

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Modelling the global climate response to orbital forcing and atmospheric carbon dioxide changes

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At least part of the record of climate change during the past million years may be attributed to forcing by changes in the geometry of the Earth's orbital system. Several attempts have been made to model the record of climate change (usually represented by the record of global ice volumes as recorded in marine sediments) using astronomical forcing^{1,2}. These models have, in general, concentrated on the direct response of the ice sheets to changes in solar insolation resulting from orbital variations. So far no fully successful simulation of the climate record has been made. We investigate here the operation of a model which is forced simultaneously by changes in the distribution of solar insolation caused by orbital variations and by changes in the atmospheric carbon dioxide (CO₂) concentration which are themselves forced by orbital parameters. The model results show the potential importance of other components in the climate system and their associated response to changing orbital parameters.

Hays *et al.*³ were the first to show that spectral analysis of climate records recovered from deep-sea sediment studies reveals concentrations of variance at orbital frequencies, and that the relative contributions of the orbital components of eccentricity, tilt and precession varies among different parts of the climate system. Most of the effort to relate orbital changes to climate has gone into the simulation of the Northern Hemisphere ice volume record rather than of temperature records of

more local significance. Borecker⁴ examined the record implied by glacio-eustatic sea-level variations recovered from studies on the island of Barbados⁵ and by deep-sea core studies. He concluded that a combination of the orbital elements such as perceived in summer at 45° N provided the best simulation of the record; this may be contrasted with the earlier conclusion of Milankovitch⁶ that a latitude of 65° N would represent the most sensitive region for control of the great ice sheets of the Pleistocene.

These early results were restricted to the past 130 kyr and do not, in fact, satisfactorily simulate longer records. As shown by Hays *et al.*³ the dominate frequency in all long ice volume records (and in most other climate records) is a periodic component near 100 kyr, the period of changes in the eccentricity of the orbit. The presence of this dominant component is not expected from the simple Milankovitch hypothesis because this period is not represented to a significant extent in the insolation record of any latitude or season⁷.

It has been suggested that the origin of this long-period component in the climate lay in the non-linear response of ice sheets to forcing at the precessional component of the orbit. As the precession effect, with a 23 kyr period, is amplitude-modulated by eccentricity, some simple non-linear systems are capable of generating power at the 100 kyr modulating frequency. Imbrie and Imbrie⁸ followed this line of reasoning and constructed a simple model which generates a reasonable ice volume simulation by forcing an ice sheet which has an exponential time-constant of growth of 22 kyr and a rate of decay with a time-constant of 12 kyr. Such a model stems from the studies of ice sheet dynamics by Weertman⁹.

The output of the Imbrie and Imbrie model is examined in spectral form in Fig. 1, where it is compared with the spectrum of the orbital input to the model (solar insolation in July at 65° N) and of the $\delta^{18}\text{O}$ record (from deep-sea core V19-30 in the equatorial Pacific¹⁰) which it aims to simulate. As shown in Fig. 1, whereas the spectrum of the model output contains significant variance at all orbital frequencies, the distribution of variance does not resemble the geological records—the model contains too much variance in the precessional band and not enough at the long period eccentricity frequency. In addition, cross-spectral comparisons of the $\sigma^{18}\text{O}$ record and the model output shows that the phase relationship at the eccentricity period is not well simulated. Partly for these reasons, the time series output of the model is not fully satisfying. Note also that the spectra shown in Fig. 1 are calculated over a time period of 340 kyr and that over the time span of the past 730 kyr, the model contains a significant 400-kyr period which is not observed in the ice-volume data records⁸.

To evaluate where this simple model fails to simulate the climate we consider the equation set which describes the model:

$$\frac{dy}{dt} = \begin{cases} \frac{(1-b)}{T_m} & x \leq y \\ \frac{(1+b)}{T_m} & x > y \end{cases}$$

where y is the ice-volume, x the solar insolation forcing function, T_m the mean time-constant of the ice sheet and b the degree of non-linearity in the rate of growth versus decay of the ice sheets. Thus, the model states that the rate of ice sheet growth and decay is (almost) linearly related to the changes in solar insolation. In terms of the variance spectrum, we would expect the solar forcing spectrum to be similar to the spectrum of the time derivative of the ice volume record. Figure 1d shows the spectrum of the time derivative of the $\delta^{18}\text{O}$ record of core V19-30 which can be compared with the solar insolation spectrum for 65° N during July shown in Fig. 1a. Apart from the lack of forcing at 100 kyr, Fig. 1 shows that the solar insolation spectrum is very similar to the ice volume derivative, and reinforces a widely-held view that a forcing at 100 kyr must be found¹¹.

One potential forcing to global climate change may be changes in atmospheric CO₂. It has been shown that changes in the

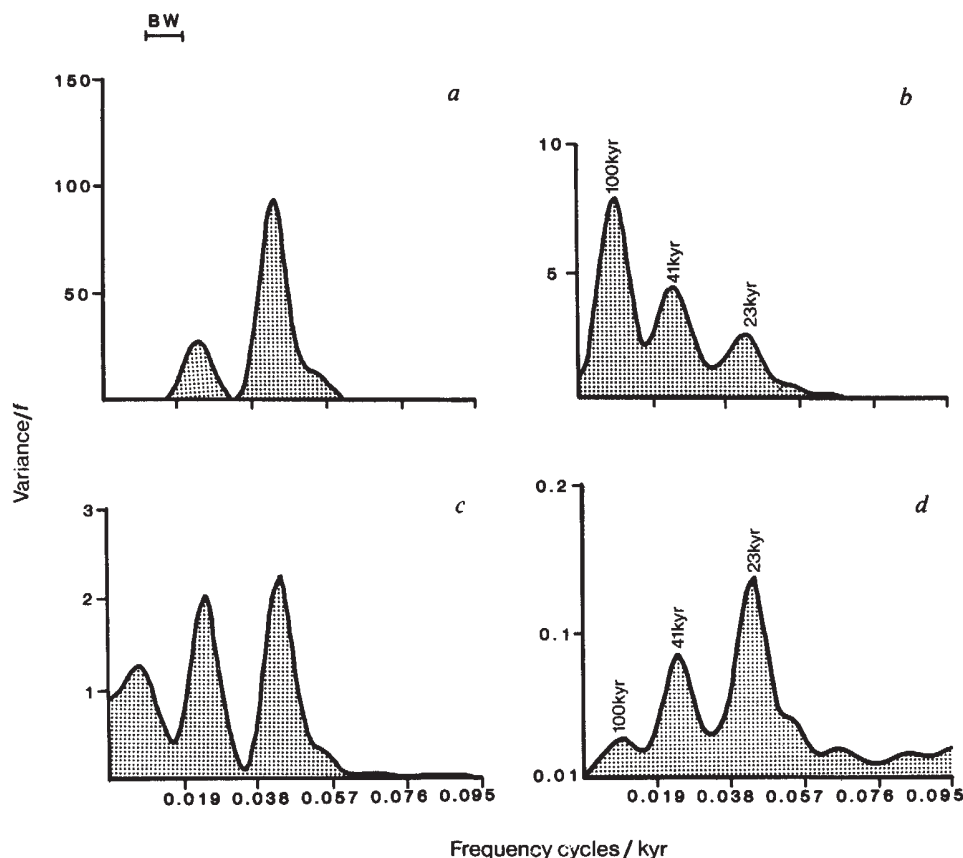


Fig. 1 Linear variance spectra for: *a*, solar insolation at 65° N in June; *b*, the simulated ice volume from the simple ice-sheet model of Imbrie and Imbrie⁸; *c*, the benthic oxygen-isotope record from core V19-30; and *d*, for time derivative of the 340-kyr benthic oxygen-isotope record from core V19-30. All spectral calculations are from 326 data points spanning the past 340 kyr. Confidence intervals (80%) and bandwidths shown for calculations from 120 points of sample auto-covariance functions. Important periods associated with orbital parameters are labelled.

concentration of carbon dioxide in the Pleistocene atmosphere^{10,13} were of sufficient magnitude to have had a significant effect on global climate. Broecker¹⁴ has suggested that the increase in atmospheric CO₂ of about 100 p.p.m. at the end of the last glacial stage may have provided an important amplification which contributed to the very rapid decay of the Northern Hemisphere ice sheets. Broecker argues that as the sea level rose because of ice sheet melting, deposition of organic carbon (and associated phosphate) on continental shelves resulted in an increase in atmospheric CO₂. The increased CO₂ would, in turn, contribute to ice-sheet decay through the greenhouse effect. However, it has been shown that¹⁵, using the Broecker model, changes in CO₂ concentration occur before changes in ice volume over the whole orbital frequency band including the 100 kyr period. The Broecker model¹⁴ assumes that the gradient of $\delta^{13}\text{C}$ between the deep ocean and the warm surface ocean reflects atmospheric CO₂, and holds true for the past 30 kyr (ref. 10). Our previous work provides continuous records of both ice volume (benthic $\delta^{18}\text{O}$) and CO₂ (planktonic minus benthic $\delta^{13}\text{C}$) changes for the past 340 kyr from equatorial Pacific core V19-30. Thus, the results of the detailed study of data from V19-30 suggests that the changes in atmospheric CO₂ are part of the forcing of ice volume changes. Of importance to the modelling problem discussed above, the variance spectrum for the carbon isotopically derived CO₂ record from core V19-30^{10,15} shows that a significant amount of variance is contained at 100-kyr periods (Fig. 2).

To test the hypothesis that CO₂ variations are indeed part of the forcing of ice volume changes¹⁵ and provide the necessary forcing at 100-kyr periods, we have added the CO₂ record from core V19-30 to the forcing function of the simple ice model of Imbrie and Imbrie⁸. The amount of CO₂ forcing added to the solar insolation at 65° N was adjusted so that the spectrum of input to this new model has the same form as the time derivative of the benthic $\delta^{18}\text{O}$ record from core V19-30 (Fig. 1*d*). The carbon, isotopically-derived CO₂ proxy makes up 22% of the variance in the model input.

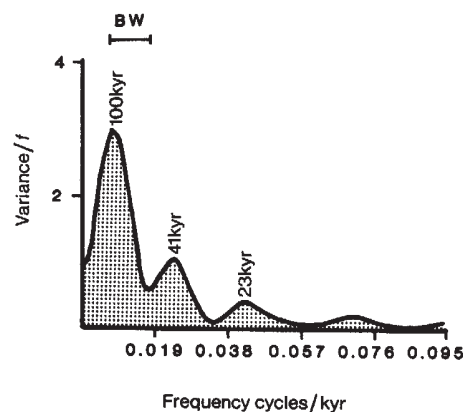


Fig. 2 Variance spectra of atmospheric CO₂ record derived from the carbon isotopic difference between planktonic and benthic foraminifera found in core V19-30.

In the frequency domain, the simulated ice volume record obtained from the CO₂ and orbitally forced model produces two important results: (1) the distribution of variance in the model is essentially identical to the observed ice volume data throughout the Milankovitch frequency band; and (2) the phase between the model and the $\delta^{18}\text{O}$ record is much improved when compared to the model with solar insolation as the only input component (Fig. 3). More importantly, the time domain character of the CO₂ model shows an extremely good fit to the geological record (Fig. 4). Figure 4 compares the ice volume record from core V19-30 with the model outputs with and without CO₂ forcing. Note first that including the CO₂ forcing in the input greatly improves the model's ability to duplicate the relative amplitudes of the major interglacial peaks (125, 210 and 330 kyr). Also, the model without the CO₂ forcing tends to

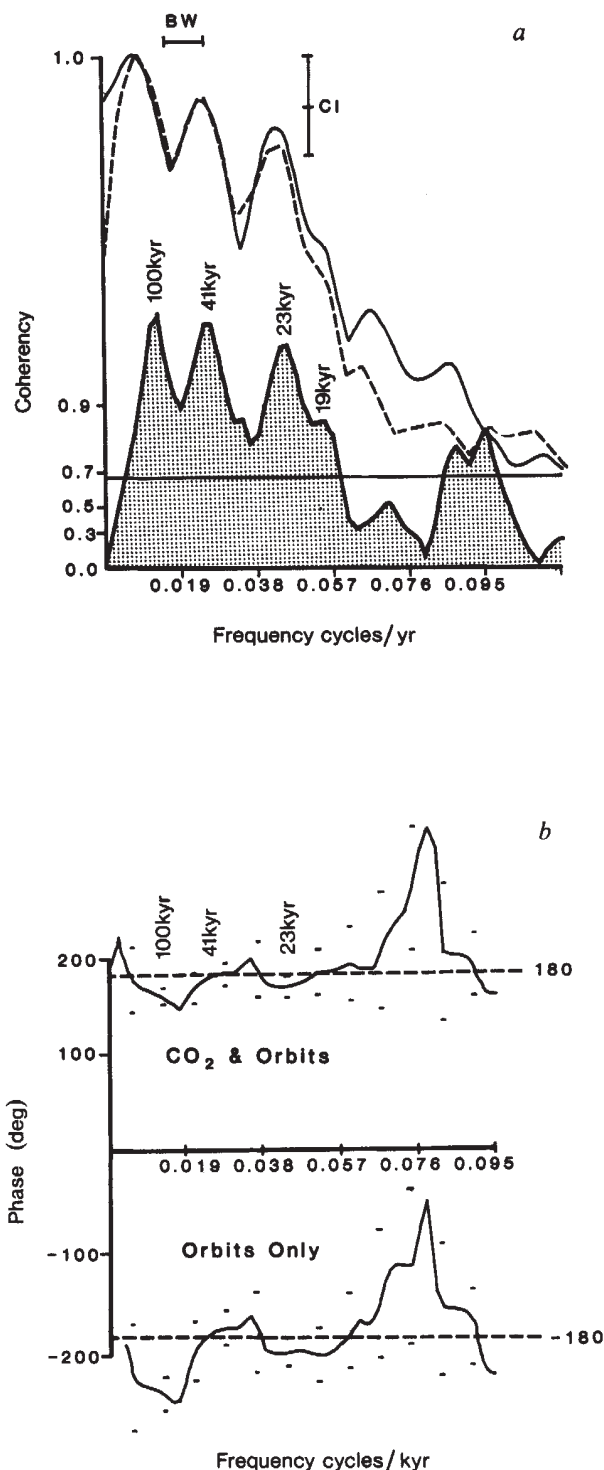


Fig. 3 *a*, Spectra for the CO₂ and orbitally-forced ice sheet model (solid line), the benthic oxygen isotopic record from core V19-30 (dotted line) and the coherency between them (shaded curve). Spectra plotted on log-scale. Horizontal line gives 80% confidence level for non-zero coherency and CI gives the 80% confidence for coherency estimates significantly different from zero. Coherencies are plotted on a hyperbolic arctangent scale. Note the almost perfect fit at all orbital frequencies and the very high coherencies. *b*, Phase spectra between the orbital models output with and without CO₂ forcing and the geological data. The expected phases should be 180° since oxygen isotopic values are inversely related to ice volume. Dashes above and below curves give 95% confidence intervals for phase estimates.

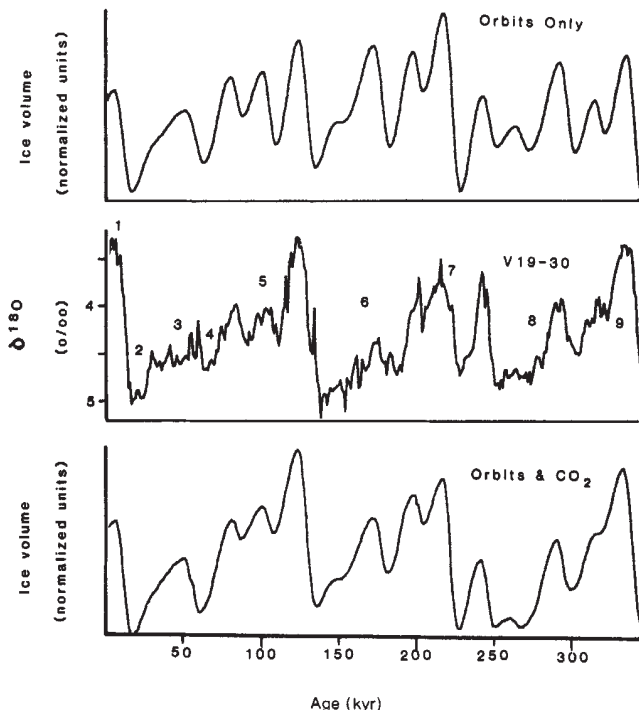


Fig. 4 Time domain plots for the benthic oxygen isotope record from core V19-30, the CO₂ and orbital model output and the ice-sheet model with orbital forcing only.

underestimate ice volume in Stage 6 (170 kyr) and Stage 8 (240–300 kyr). Also, the amplitude of the substages in Stage 5 are better depicted in the CO₂ model. Thus, we feel that inclusion of the carbon-isotope derived CO₂ record significantly improves the ability of the simple ice growth and decay model of Imbrie and Imbrie to simulate changes in global ice volume over the past 340 kyr.

We have used a simple model to test the hypothesis that CO₂ variations are an important component in Pleistocene climate; the next step would be to use more physically realistic models such as presented by Birchfield *et al.*¹ or Pollard². However, our results strongly suggest that other components of the climate system must be considered in modelling ice volume changes. Previous modelling efforts have concentrated on ice-sheet dynamics in response to direct orbital forcing. Our results indicate that orbital changes and the oceanic response to insolation changes may also have an important role in affecting climate conditions and the growth and decay of large Northern Hemisphere ice sheets.

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Major shear zones and autochthonous Dalradian in the north-east Scottish Caledonides

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The Buchan area within the Grampian Caledonides of north-east Scotland is a classic locality for the study of low-pressure regional metamorphism. Using detailed geophysical surveys, pits and boreholes to augment sparse outcrop data, we have revealed a system of steep shear belts of Grampian age. When combined with a reassessment of the stratigraphy, the new information leads us to the view that the Banff nappe does not exist. We also argue that the Tay nappe is of limited extent in Buchan, and that the tract of uninverted Southern Highland Group (upper Dalradian) is autochthonous.

In the Buchan area, the metasediments of the Southern Highland Group largely lie the right way up in a shallow syncline^{1,2}. They pass downwards, with increasing grade of metamorphism of classic 'Buchan' type³, into Argyll Group rocks⁴. The boundary coincides roughly with a transition from andalusite schists to migmatitic gneisses.

Read¹ proposed that his Banff Division (mainly Southern Highland Group) was equivalent to the uninverted limb of the Tay nappe, but separated from its migmatitic core by the Boyne lag surface with the rocks above this surface constituting the Banff nappe. Excision along the Boyne lag was invoked to explain the apparent absence, in north-east Scotland, of rocks now placed in the Tayvallich Subgroup (uppermost Argyll Group⁴). More recently, Ramsay and Sturt have suggested⁵, that the gneisses below the Boyne lag are exotic slabs of Cadomian basement forming the base of a larger, composite Banff nappe⁶, thrust northwards over the Tay nappe on a basal thrust, the Portsoy thrust. The Boyne lag would then represent a surface of basement-cover detachment developed during nappe emplacement. On the other hand, stratigraphical continuity has been postulated^{4,7,8}, and the apparent local absence of the Tayvallich Subgroup has been attributed^{8,9} to lateral facies variation.

Since 1970 we have been engaged in a general re-investigation of the Buchan area during which we discovered a regional system of shear belts consisting of steep anastomosing, large and small-scale shear zones (Fig. 1).

These shear zones have experienced an extensive history of igneous intrusion including the 'Younger Basics'; later minor diorites, granodiorites and granites; and local concentrations of still later muscovite-biotite granite sheets and associated tourmaline pegmatites, dated by the Rb/Sr method at ~465 Myr (refs 10-12). The Younger Basic intrusions are frequently severely displaced and disrupted, exhibiting confused igneous stratigraphy, upturned layering, and local stripping of marginal hornfels¹³⁻¹⁷. The mylonite fabrics are generally steeply inclined and magnetic units within the shear belts are modelled as steeply dipping slabs^{14,15,17}. Infrequent extension lineations, generally plunging steeply down-dip, are defined by deformed detrital grains, porphyroblasts and metamorphic segregations in metasediments; and elongate mineral aggregates in igneous rocks.

The continuity of the shear belts has been demonstrated by magnetic surveys and confirmed by direct investigation in pits and trenches. The observed fabrics postdate the Younger Basics (490 Myr)²⁹. Basic mylonites are cut¹⁵ by members of the 465-Myr pegmatite suite which are themselves strongly deformed locally^{15,18}. Palaeomagnetic observations on the Younger Basics are consistent with lower Ordovician pole positions¹⁹ and show that the main disruption occurred while they were still above magnetite blocking temperatures (~550 °C). It seems likely that shearing commenced shortly after emplacement of the Younger Basic suite, probably during D3 of the regional chronology (481 ± 15 Myr)²⁰.

A wide range of mineral assemblages has been noted in the varied rock types found in the shear zones. Blastomylonitic derivatives of pelitic rocks commonly contain fibrolite while calc-silicate rocks show coexisting calc-amphibole and diopside; ferromagnesian minerals in the sheared mafic igneous rocks are replaced by aggregates of fine grained amphibole and biotite. The mineral assemblages imply maximum temperatures of 600 °C and pressures of 2-3 kbar (ref. 21), indicative of low-pressure amphibolite facies metamorphism.

Within the Dalradian, this style of deformation is apparently unique to the Buchan area. The shear zones' control over the

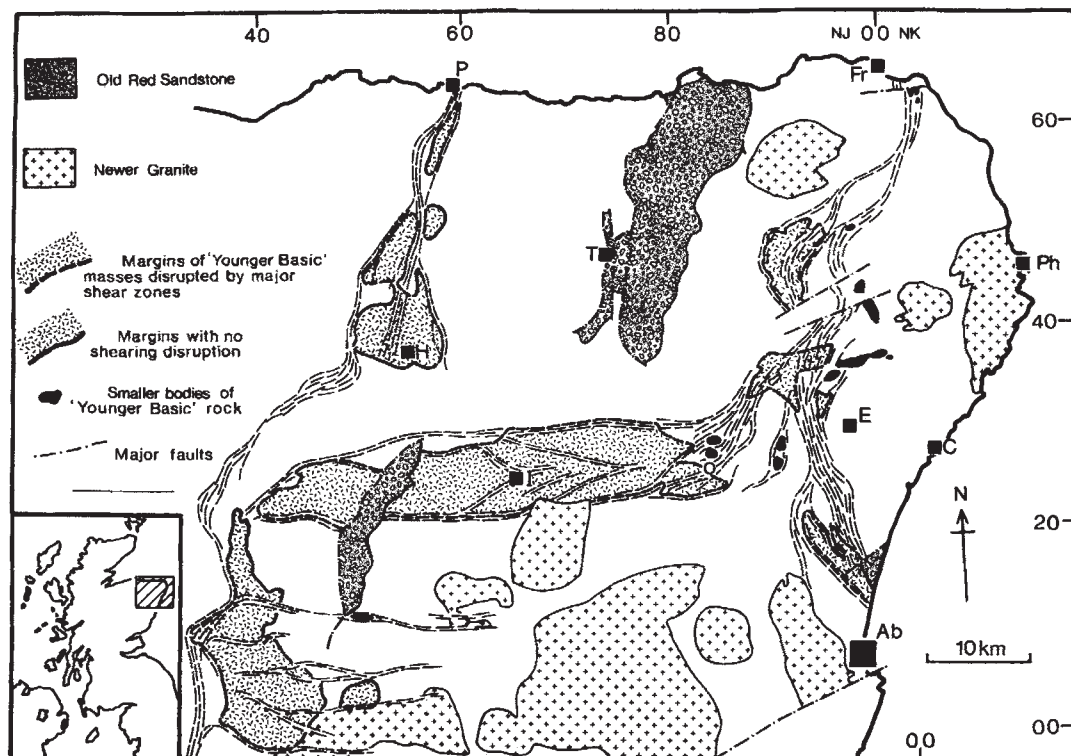
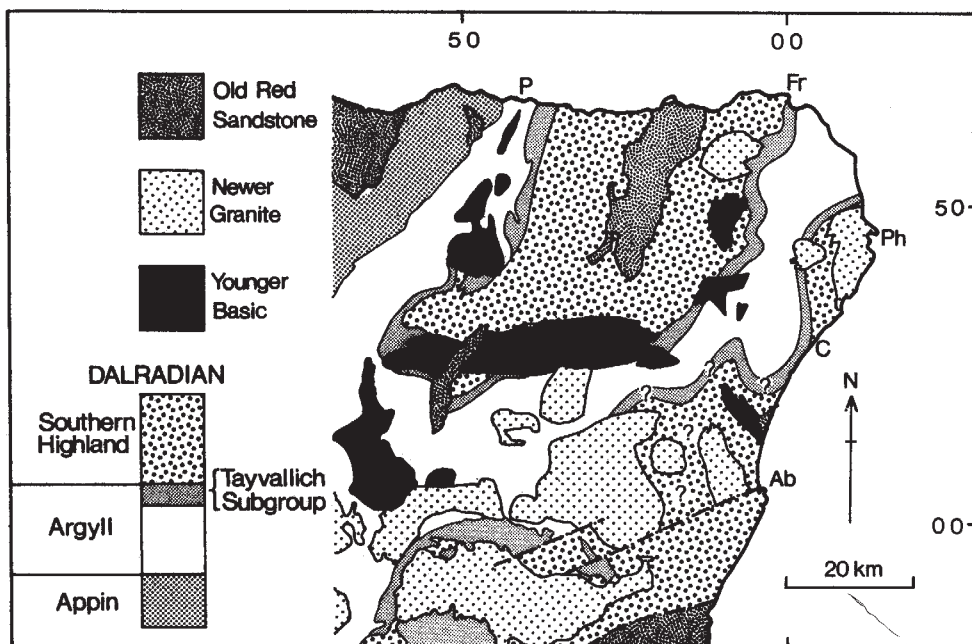


Fig. 1 Distribution of major shear belts in north-east Scotland. Also shown, principal younger basic (synorogenic) masses, newer granites (post-orogenic), and Old Red Sandstone (Devonian molasse). Ab, Aberdeen; C, Collieston; E, Ellon; Fr, Fraserburgh; H, Huntly; I, Inch; O, Oldmeldrum; P, Portsoy; Ph, Peterhead; T, Turrieff.

Fig. 2 Dalradian stratigraphy in north-east Scotland. Also shown, Younger Basics, Newer Granites, Old Red Sandstone. Localities lettered as in Fig. 1. Based on Aberdeen University/BGS contract mapping; refs 2, 8.



emplacement of the Younger Basics suggests that they extend for a considerable depth. The western (Huntly/Portsoy) belt appears also to have channelled 'Older' (pre-metamorphic) basic intrusions. Furthermore, it roughly coincides with the inferred western limit of deep-water sedimentation in upper Argyll and Southern Highland Group times. We suggest that the western belt has a long tectonic history, perhaps initially as a zone of major extensional faulting²²⁻²⁴, and may not have moved significantly relative to basement since Dalradian sedimentation.

Our work confirms the stratigraphical continuity implied by Fettes and Harte (in ref. 4) and Harte⁸ between Fraserburgh and Oldmeldrum and we thus support, with modifications (Fig. 2), the stratigraphical correlation proposed by Fettes and Harte⁴. The effects of the shear belts, of previously unrecognized faults on an ENE trend, and exceedingly poor exposure tend to obscure the continuity of the Tayvallich subgroup (which constitutes the only distinctive horizon in a thick clastic turbidite succession), and the essential stratigraphical, structural and metamorphic coherence of the Dalradian succession (see Fig. 2).

The transition to migmatitic gneisses is usually the result of a progressive increase in the grade of regional metamorphism although high- and low-grade rocks may locally be juxtaposed by the shear zones. Furthermore, rocks with a gneissose aspect may also result from deformation in the shear zones. Sturt *et al.*⁵ present scattered isotopic data apparently indicating a 700-Myr age of metamorphism for the gneisses. We suggest that the Rb/Sr systematics implied by the scatter are too complex to permit straightforward interpretation and that the 'isochrons' probably reflect components of provenance, sedimentary fractionation, diagenesis and Caledonian metamorphism.

Our observations directly indicate that there is no longer any need for a Banff nappe: there is no major break in the stratigraphy and no sign of major, low-angle thrusts on the ground.

Our new observations on the Buchan area reveal it as being distinctive in the Scottish Dalradian outcrop. The region has a gravity high of about +15 mGal with steep gravity gradients into lows of the order of -50 mGal to the west and south²⁵. The steep gradients coincide with major shear belts. The gravity high extends some 40 km offshore to the east, and points to an absence of major bodies of granite in the crust. It is probably caused by high densities in the Dalradian metasediments for which we have measured values in the range 2.75-2.80 g cm⁻³.

Post-orogenic uplift of the Buchan area implied by metamorphic mineral assemblages has only been about 10 km, in contrast to estimates of up to 30 km in the intermediate pressure zones to the west and south. The area also responded differently to

tension and dolerite intrusion in late Carboniferous times, allowing the emplacement of numerous dykes.

We interpret these observations as indicating that the Buchan area is a distinctive crustal segment, partly decoupled from the terranes to the south and west by relative (largely vertical) motion on the shear belts. The west margin of the area is defined on both geological and gravity criteria as the steep zone and serpentine belt that extends from Portsoy to the SSW. The south margin is less well-defined since there are several shear zones more widely spaced, and stratigraphy and structure are seriously obscured by late granites. We envisage large displacements (at least 5 km) across the southern and western shear belts, with the Buchan area on the downthrow side throughout. The Fraserburgh-Oldmeldrum shear belt is seen as dividing the Buchan area into two sub-areas which have only jostled against each other without much overall displacement. Within the Buchan area, pre-Grampian structures may well be more influential in subsequent deformation than appears to be the case elsewhere in the Scottish Dalradian.

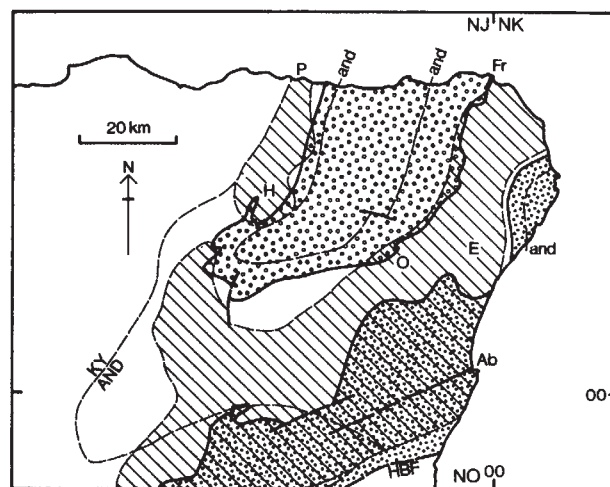


Fig. 3 Outline of metamorphism and structural disposition in eastern Dalradian. Southern Highland Group of nappe belt, fine stipple, (also includes upper Argyll Group of Tarfside nappe). Right-way-up Southern Highland Group of Buchan (autochthonous?), coarse stipple. Also shown: sillimanite zones (oblique hachure); andalusite isograd (and); kyanite/andalusite inversion (KY/AND). Based in part on refs 2, 8, 26, 27. Localities lettered as in Fig. 1. HBF, Highland Boundary Fault. Metamorphic peak is in post-D1, pre-D3 interval.

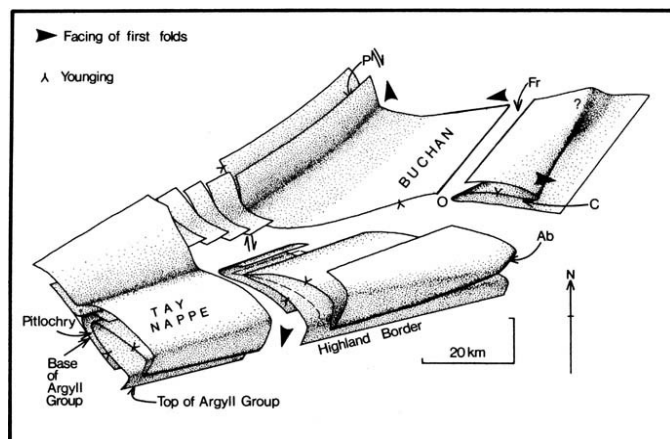


Fig. 4 Block diagram illustrating the relations of the primary structures in the north-east Dalradian. Based in part on ref. 28 and other published sources.

In disputing the reality of the Banff nappe, we are obliged to search for other solutions to the status of the Buchan Dalradian. Perhaps the simplest alternative would entail a northeastwards continuation of the Tay nappe, with an implied increase in amplitude to accommodate the Buchan Dalradian outcrop, which would then represent the uninverted limb of the structure. However, the apparent northeastwards decrease in pressure experienced by rocks at a low structural level in the nappe belt (marked by the appearance of andalusite and cordierite, see Fig. 3) argues for a decrease in the significance of nappe structures in this direction. The Southern Highland Group metasediments of the Collieston coast section could reasonably be considered on structural grounds as a strike continuation of the structures at the southeastern limit of the nappe belt. However, in uninverted rocks of the Southern Highland Group to the west of the Fraserburgh–Oldmeldrum belt (Fig. 3), F1 folds face westwards, in contrast to the ESE facing folds of the Collieston section. The preservation of much primary detail (in contrast to Collieston and the nappe belt), supports the impression of a different structural regime.

The moderate uplift, paucity of granite and presence of a positive gravity anomaly argue for relatively minor crustal thickening in this region during the Grampian orogeny, in contrast to the nappe belt to the south. This suggests that any northeastwards continuation of the nappe structures must involve a decrease in amplitude, and so be confined to the southeastern part of the Buchan area (see Fig. 4). Although these ideas developed independently in the course of our work, B. Harte (personal communications) has also suggested that the Tay nappe may decrease in amplitude from the Central Highlands to the Buchan area.

In rejecting hypotheses involving a distinct Banff nappe, or a correlative of the Tay nappe, we are driven to adopt the simplest solution which is consistent with the geological and geophysical data. We propose, therefore, that the Buchan Dalradian is autochthonous, to the extent that it is now underlain by the basement on which it was deposited. This does not exclude the possibility of wholesale imbrication of extensionally thinned crustal segments, or of a major decollement at or near the base of the crust.

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Evidence against large-scale Carboniferous strike-slip faulting in the Appalachian–Caledonian orogen

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Kent and Opdyke¹ have argued that during the late Devonian and early Carboniferous, the eastern part of the northern Appalachians (Acadia) was situated ~1,500 km further south relative to cratonic North America than it is at present, and that it moved to its present position sometime later in the Carboniferous (Fig. 1). Van der Voo and Scotese² proposed an even greater displacement of 2,000 km which they attribute to motion between cratonic North America and Europe, Acadia being attached to Europe (Fig. 1). The argument is that palaeolatitudes (λ_p) derived palaeomagnetically from Upper Devonian and Lower Carboniferous rocks of cratonic North America are 15–20° more northerly than those of Acadia and Europe. The proposed shear zone passes through central Newfoundland. Using palaeomagnetic results from eastern and western Newfoundland we show here that no such motion occurred. We also show that Kiaman (late Carboniferous and Permian) overprinting is widespread in Newfoundland, and that these secondary magnetizations agree (with two exceptions attributed to dextral rotation of the Colorado Plateau) with observations from late Devonian and early Carboniferous rocks of the North American craton, confirming the proposal³ that magnetizations of the cratonic early Carboniferous rocks are Kiaman, not early Carboniferous in age. Our results also enable us to extend this proposal³ to late Devonian rocks of the craton. Hence the palaeolatitudinal offset of Fig. 1 is almost certainly not tectonic, but is an artefact of the wrong assumption of the equivalence of rock and magnetization ages.

The hypothesis⁴ of global Kiaman overprinting of magnetizations has been recently revived⁵, and shown to be physically and geologically plausible. The Kiaman palaeofield was almost continuously reversed⁵, so that uniform secondary magnetization could have been acquired over a long time without the complications of reversals. The Kiaman was a time of unprecedented continentality, with a huge supercontinent (Pangaea) astride the palaeoequator⁶; rainfall was seasonal and the water-table depressed, and secondary haematite, capable of retaining a stable chemical remanent magnetization (CRM)⁷ was a common product. Mild heating, during the accumulation of thick rock sequences at the time of the Hercynian–Appalachian orogeny, could have converted oxyhydroxides of iron to haematite and produced a stable CRM⁸. Also, uplift and cooling following deep burial could have caused the acquisition of viscous partial thermo-remanent magnetization (VPTRM)^{9–11}.

Ninety-seven per cent of magnetizations observed in cratonic rocks of late Devonian and early Carboniferous age from North America are reversed, having southerly directions with near-

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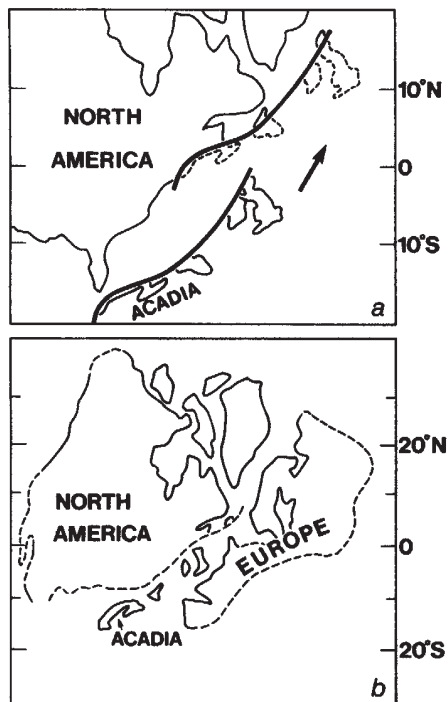


Fig. 1 Hypothesis of large-scale Carboniferous transcurrent motion. *a*, The proposed displacement of Acadia redrawn from ref. 1. *b*, The proposed displacement of North America relative to Europe and Acadia (note that Europe is broken in the middle) modified from ref. 2. Palaeolatitude scales are on the right.

equatorial inclinations^{12,13}. This should be compared with 95% reversed magnetizations observed in Kiaman rocks globally¹⁴. There are a few intermediate negative inclinations, notably (about -40° , $\lambda_p = 23^\circ\text{S}$) in the Mauch Chunk Formation of Pennsylvania¹⁰. These are truly univectorial magnetizations, unchanged up to 650°C . The predominant southerly, reversed, low-inclination directions have generally been accepted as representative of the cratonic palaeofield^{1,2,15}. But are the cratonic observations (1) a true record of the late Devonian–early Carboniferous field and do the very rare more steeply inclined normal directions merely reflect short-term excursions, or (2)

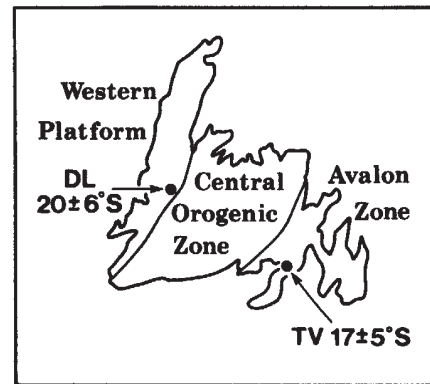


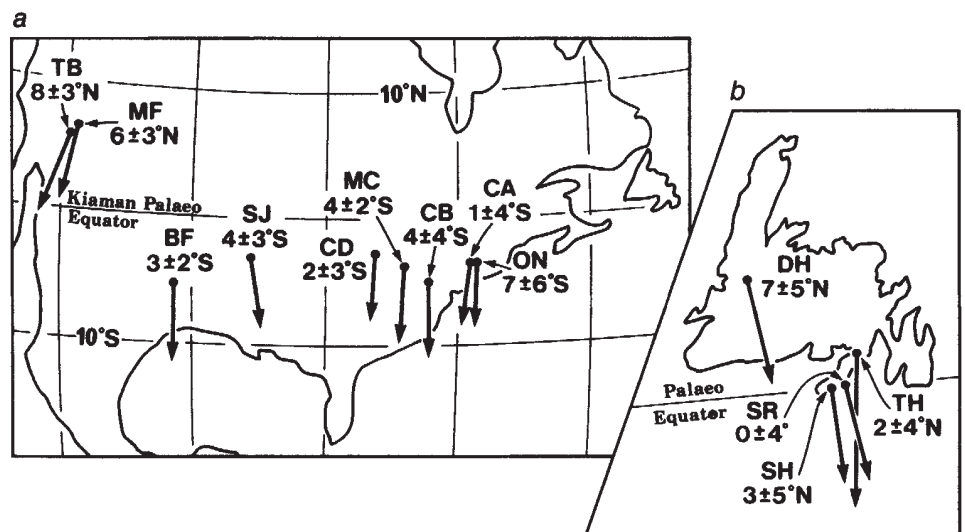
Fig. 2 Comparison of palaeolatitudes observed from pre-folding magnetizations in Lower Carboniferous rocks of the western platform of Newfoundland (DL, Deer Lake Group, Viséan^{12,18,19}) and the Avalon Zone (TV, Terrenceville Formation, Tournaisian^{12,21}).

are they Kiaman overprints, the less common normal magnetizations representing the only surviving record of the original field? The hypothesis of large Carboniferous displacement is justified only if (1) is true.

Until recently, studies of cratonic rocks of late Devonian or early Carboniferous age have all been from the US, and only in one has it been concluded¹⁶ that Kiaman overprinting is present (Onondaga limestone). That only one instance should have been recognized is surprising because studies of Palaeozoic rocks of other ages from cratonic North America commonly record evidence of it^{10,11,17}.

We have recently made the first study of a cratonic rock-unit (Viséan Deer Lake Group) in this age range in Canada^{12,13,18,19}. These red beds lie on the Precambrian western Newfoundland platform, which is part of the Canadian Shield, and are part of cratonic North America. They have a pre-folding Viséan magnetization of both polarities (declination, $D = 355^\circ$; inclination, $I = -36^\circ$; $\alpha_{95} = 11^\circ$). The palaeolatitude ($\lambda_p = 20 \pm 6^\circ\text{S}$) is consistent with that obtained from the very stable magnetization observed from the cratonic Mauch Chunk Formation¹⁰, but inconsistent with most cratonic observations from the US. We have identified and separated a secondary magnetization ($D = 158^\circ$, $I = -13^\circ$, $\alpha_{95} = 10^\circ$, $\lambda_p = 7 \pm 5^\circ\text{N}$) which indicates that our result has no overprints. Textural studies show that secondary

Fig. 3 Observations from Upper Devonian and Lower Carboniferous rocks compared with the early Kiaman (latest Carboniferous and early Permian) field. *b*, Overprints observed in four rock-units from Newfoundland compared with the Kiaman palaeoequator. DH, Deer Lake Group overprint, Viséan¹²; SH, St Lawrence Pluton overprint¹³; Spanish Room Formation, Permo-Carboniferous¹³; Terrenceville Formation overprint, Tournaisian²¹. *a*, Observations from Upper Devonian and Lower Carboniferous cratonic rocks are compared with the Kiaman palaeolatitude grid. The results are as follows: BF, Barnet Formation, Lower Carboniferous²⁶; CA, Catskill redbeds, flat-lying, Upper Devonian¹; CB, Catskill redbeds, folded²²; CD, Columbus and Delaware limestone, Devonian²⁷; MC, Mauch Chunk Formation, Viséan¹⁵; MF, Martin Formation, Upper Devonian²⁸; ON, Onondaga Limestone, Lower to Middle Devonian¹⁶; SJ, St Joe limestone, Tournaisian²⁹; TB, Temple Butte Formation, Upper Devonian²⁸. A poorly observed result²⁷ from the Raisin River Dolomite is not included: its inclination agrees with the Kiaman field¹⁵. The early Kiaman grid is calculated using the palaeopole (44°N , 126°E , $A_{95} = 4^\circ$) derived from cratonic results ranging in age from late Westphalian to Sakmarian (mean ~ 280 Myr) close to the Permian–Carboniferous boundary³⁰. The accuracy of the palaeolatitudes of the early Kiaman grid recently has been confirmed from Ellesmere Island³¹.



haematite, probably related to groundwater circulation, is present²⁰, providing the means by which the overprint was acquired.

The Terenceville Formation (Tournaisian) of the Avalon Zone, situated on the opposite margin of the Appalachian orogen²¹, also contains both a prefolding magnetization ($\lambda_p = 17 \pm 5^\circ\text{S}$ (ref. 12)) with normal and reversed polarities, and a post-folding overprint ($\lambda_p = 2 \pm 3^\circ\text{N}$). The former is in excellent agreement with our result from the western Newfoundland platform (Fig. 2). The Viséan Deer Lake Group and the Tournaisian Terenceville Formation are not exactly coeval and the errors are not insubstantial, so the observations do not preclude motion, although they show that very large displacements, such as those invoked in Fig. 1., have probably not occurred.

We have also observed two other overprints in rock units of the Avalon Zone, in the Carboniferous Spanish Room Formation and in the latest Devonian St Lawrence Pluton¹³. Both give near-equatorial palaeolatitudes. All four Newfoundland overprints agree with the early Kiaman field (Fig. 3) and are, therefore, probably of this age.

Observations from late Devonian and early Carboniferous rocks of cratonic North America also agree with the Kiaman field (Fig. 3). All yield near-equatorial palaeolatitudes and all agree with Newfoundland overprints. Particularly impressive is the exact agreement of the most detailed studies (MC, CA, CB, ON of Fig. 3) with the Kiaman field. Results TB and MF show small departures of declination (Fig. 3), which, we suggest, record a post-Permian clockwise rotation ($15 \pm 4^\circ$) of the Colorado Plateau. Everywhere, inclinations are in excellent accord with the early Kiaman field.

Arguments have been made to support the assumption (essential to the hypothesis of large displacement) that these cratonic observations truly record the late Devonian–early Carboniferous field, especially fold-tests for the Catskill and Mauch Chunk redbeds (Fig. 2, CB²², MC¹⁵). The magnetization predates the Alleghanian (loosely dated as late Carboniferous or Permian) folding. Because it is parallel to the early Kiaman field, we argue that the magnetization is Kiaman and immediately predates the folding. A recent study⁸ shows that certain folded Silurian beds in the southern Appalachians acquired their magnetization by the transformation of goethite to haematite at about 80°C during burial in the late Carboniferous just before Alleghanian folding. A similar process could have produced the predominant (and, we argue, secondary) magnetizations of the Mauch Chunk and Catskill redbeds. Alternatively they could be uplift VPTRMs^{9,10}.

Three points are clear. First, early Carboniferous results from either side of the Appalachian orogen in Newfoundland show no displacement (Fig. 2). Second, all observations of inclination (palaeolatitude) from Upper Devonian and Lower Carboniferous rocks of the craton agree with the much younger Kiaman palaeofield, and with known Kiaman overprints (Fig. 3); the predominant southerly-directed near-equatorial magnetizations of the craton probably do not record the palaeofield at the time the rocks were deposited, but at ~ 50 Myr later. Third, palaeolatitudinal determinations from the craton that are known to be free from Kiaman overprinting, indicate southerly palaeolatitudes of about 20°S for middle North America, consistent with those from Acadia and Europe (Fig. 1). Hence, there is now no basis for invoking large-scale displacement of Acadia and Europe relative to cratonic North America during the Carboniferous. The apparent palaeolatitudinal discrepancies of Fig. 1 arose because magnetizations that are almost certainly secondary and of Kiaman age in North America have been compared with older, probably truly late Devonian or early Carboniferous, magnetizations of Acadia and Europe.

These arguments do not affect the hypothesis of smaller-scale late Palaeozoic strike-slip faulting²³. Neither do they affect the proposal²⁴ of large-scale sinistral displacement during the mid-Devonian before the events discussed here.

Since submitting this letter, an abstract has appeared outlining results of a revised study of the Mauch Chunk Formation²⁵, which has resolved its magnetization into pre-folding ($\lambda_p = 15^\circ\text{S}$) and post- or syn-folding Kiaman ($\lambda_p = 2^\circ\text{N}$) components, pro-

viding further support for our arguments and those in ref. 3.

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Mass transfer rates along vertical walls in magma chambers and marginal upwelling

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The origin of compositional zonation in magma chambers^{1–11} has created much interest. Observed chemical, mineralogical and thermal gradients in many ash flow deposits are widely believed to indicate pre-eruptive zonations within the magma reservoir^{7,12,13}. By analogy with laboratory experiments involving salt crystallization and the concomitant release of chemically-induced buoyancy, it has been suggested that compositionally evolved magma might accumulate at the top of a magma chamber. Crystallization of mafic phases along the chamber margins would produce a residual melt which is more silicic than the bulk composition. Marginal upwelling of buoyant melt could then lead to vertical compositional stratification underneath the roof of the chamber^{1,3,6,7,14–16}. Here we show that analysis of laminar boundary layer flows driven by thermal and/or chemical buoyancy indicates that, for physical parameters characteristic of magma, the rate of enriched magma accumulation is small compared with geologically-inferred rates. Furthermore, the effect of a temperature-dependent rheology is to suppress the marginal upwelling effect⁸. Even for the extreme case of an isothermal chamber, sidewall upwellings can only be quantitatively significant for highly mobile species (chemical diffusion, $D > 10^{-11} \text{ m}^2 \text{ s}^{-1}$) located in low-viscosity basaltic chambers with a high surface area-to-volume ratio.

Various possibilities for buoyancy-induced flows along the walls of a magma chamber are summarized in Fig. 1. In the simple case, where only thermal buoyancy is important (Fig.

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1a), negative buoyancy along the cold chamber wall dictates a boundary layer region of downwelling flow there. The velocity and thermal fields depend strongly on the temperature dependence of magma viscosity, and schematic velocity profiles are given for both the constant viscosity and temperature-dependent cases. In the latter case, a rheological layer of very viscous (solidified) magma is found immediately adjacent to the wall when $T_w < T_s$, where T_s and T_w represent the solidus and wall temperatures respectively. Marginal upwelling cannot occur because no chemical buoyancy is present.

The other extreme example of marginal flow is illustrated in Fig. 1c. Here, the chamber is isothermal. Chemical buoyancy may be generated by injection of components with small partial molal densities along the walls of the chamber¹, or by partial melting of country rock along chamber margins. The growth rate of a density stratified zone at the top of the chamber depends on mass transfer boundary conditions, transport properties and magma chamber size. The quantitative theory developed below shows that mass flow through marginal boundary layers is generally small compared with roofward magma accumulation rates of $10^{-2.5 \pm 0.5} \text{ km}^3 \text{ yr}^{-1}$ as inferred geologically^{2,4}.

In the more usual situation, both chemical and thermal buoyancy act simultaneously. This is the regime of countercurrent convection where a thin upwelling chemical boundary layer is embedded within a thicker downwelling thermal boundary layer (Fig. 1b). The precise nature of compositional, thermal and velocity fields is governed by the buoyancy ratio, the ratio of mass to heat diffusivity and by boundary conditions on heat and mass transfer.

The partial differential equations expressing conservation of mass, momentum, energy and concentration appropriate for steady, laminar boundary layer flow in the countercurrent regime can be related to a set of coupled ordinary differential equations by means of the similarity transformation $\eta = Pr^{1/4} q y / x^{1/4}$ where x and y represent the horizontal and vertical coordinates, respectively and q , a scaling constant is given by $q^4 = \rho g (\alpha_T (T_\infty - T_w) + \alpha_c (C_w - C_\infty)) / 4\mu\kappa$ where q remains strictly positive^{8,17,18}. The transformed set of coupled ordinary differential equations takes on the following form in which primes denote differentiation with respect to η

$$f''' + \frac{\Gamma}{1+\Gamma} \theta - \frac{1}{1+\Gamma} \phi = 0 \quad (1)$$

$$\theta'' + 3f\theta' = 0 \quad (2)$$

$$\phi'' + 3f\phi' = 0 \quad (3)$$

The following dimensionless variables have been introduced in deriving equations (1)–(3)

$$\theta = (T - T_\infty) / (T_w - T_\infty), \quad \phi = (C - C_\infty) / (C_w - C_\infty)$$

and

$$f = Pr^{3/4} \psi x^{-3/4} (4\kappa q)^{-1}$$

where ψ is the stream function and $\Gamma = \alpha_T (T_w - T_\infty) / \alpha_c (C_w - C_\infty)$ is the buoyancy ratio. The Lewis number ($Le = \kappa / D$) measures the ratio of thermal to chemical diffusion and has an important role in determining whether or not countercurrent flow is possible. The parameters α_T and α_c represent the isobaric thermal and isobaric concentration expansivities respectively. The boundary conditions are that the wall temperature and concentration are constant at T_w and C_w respectively and that the vertical velocity vanishes there. At asymptotic distances from the wall, the temperature and concentration are set at T_∞ and C_∞ and the vertical shear stress vanishes. Pr is the Prandtl number ($= \nu / \kappa$).

The two parameters governing the structure of marginal chemical and thermal boundary layers are Le and Γ . In Fig. 2, results taken from ref. 17 are plotted over the Le – Γ plane. This plot has been constructed on the basis of numerical results obtained by solution to equations (1)–(3) for $Le < 10^2$ and by the method of matched asymptotic expansions for $Le > 10^2$. For

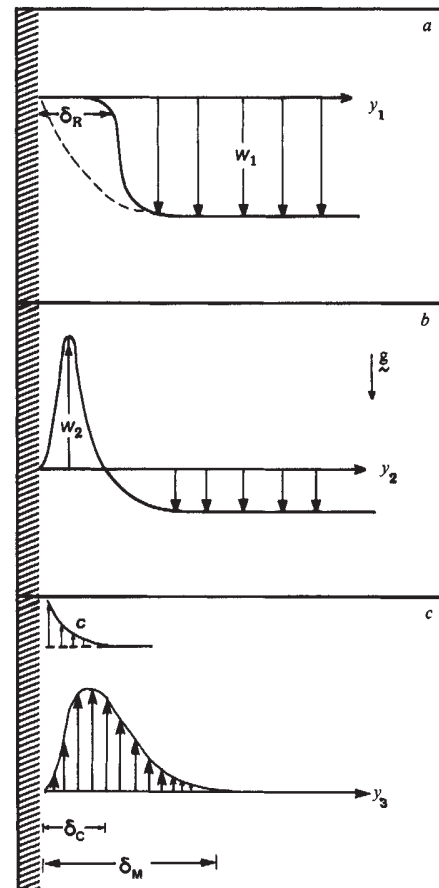


Fig. 1 Three regimes of boundary flows adjacent to a wall in a magma chamber. The vertical velocity is plotted as a function of distance normal to the wall (hatched region). The gravitational acceleration vector indicates the direction of the flow. The velocity scales w_i are different in each case. a, Descending flow structures characteristic of boundary layers driven by thermal buoyancy. δ_R indicates the thickness of the rheological boundary layer, as a consequence of a temperature-dependent viscosity. Dashed curve, the velocity profile for a constant viscosity fluid; b, counterflow situation where both thermal and chemical buoyant effects are dynamically important. This situation represents that observed in laboratory experiments^{3,6,14,15}; c, upwelling flows driven by chemical buoyancy. For diffusion coefficients of typical geochemical species, δ_c can range from centimetres to metres in thickness. The downwelling flow takes place in a region much greater than y_3 , which is based on scaling with respect to the chemical boundary layer thickness. This is an idealized case relevant to flow in an isothermal magma chamber where no thermal downwelling exists.

large Le , the asymptotes $\Gamma = 1.08 / Le^{1/3}$ and $\Gamma = 0.545 / Le$ define, the boundaries of the descending and counter flow regions and the ascending and counter flow domains, respectively. The upflow and counterflow regimes occupy a rather restricted portion of the Le – Γ plane where both the buoyancy ratio (Γ) and Lewis number are small. The locations of fast (H_2O) and slow (silica) moving species for magma of rhyolitic, intermediate and basaltic composition are shown for a range of temperature decrements across the thermal layer. Figure 2 reveals that unidirectional downward flows are expected in most cases when thermal and chemical buoyancy effects act concurrently in a chamber. A counterflow regime could be set up in a low viscosity (basaltic) chamber provided ΔT across the marginal thermal layer is small, and if the component driving the upward flow can diffuse rapidly ($D > 10^{-11} \text{ m}^2 \text{ s}^{-1}$). These conditions may be set up in large deep-seated (catazonal) mafic intrusions where, because of proximity to an underlying magma source and lack of a large heat-dissipating hydrothermal system, ΔT is small¹⁹.

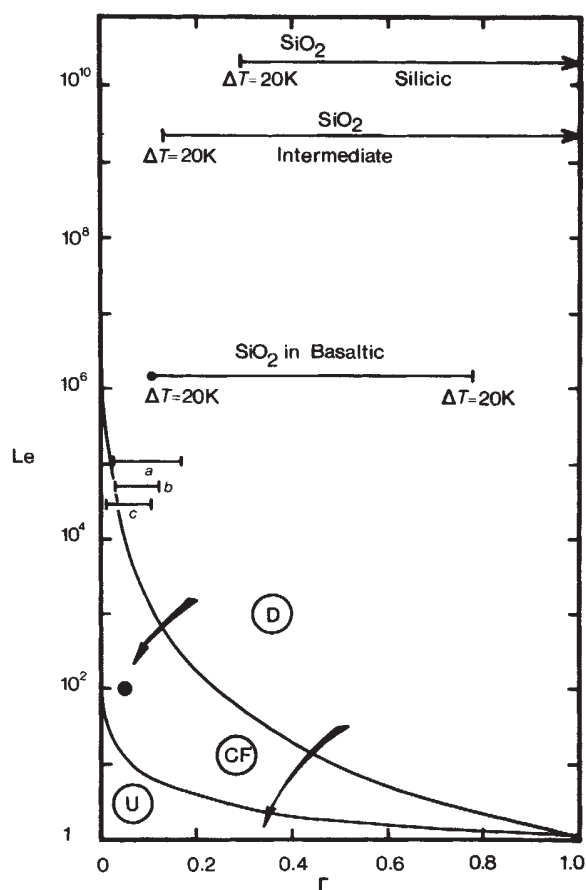


Fig. 2 Domain diagram depicting flow regimes in the Lewis number (Le) and buoyancy ratio (Γ) plane. U, D and CF denote upwelling, descending and countercurrent flows respectively. Solid circle in the countercurrent region (CF) refers to the conditions of the double-diffusion experiments in aqueous solutions¹¹. α_T equals $5 \times 10^{-5} \text{ K}^{-1}$ for all cases. ΔT is the temperature drop across the thermal boundary layer. Diffusion data for H_2O and silica in basaltic, phonolitic and rhyolitic melts taken from refs 1, 29–32, 36, 37. α_c values calculated from refs 33–35. Thermal conductivities and isobaric heat capacities are from refs 38 and 39 respectively. Properties of rhyolitic, phonolitic and basaltic melts evaluated at 850 °C, 1,050 °C and 1,200 °C respectively. This is applicable to constant viscosity melt. The influence of a temperature-dependent rheology is to suppress the countercurrent regime as indicated by the arrows⁸. Bars marked a, b, c refer to H_2O in magma of rhyolitic, phonolitic and basaltic composition, respectively.

However, for more silicic systems in which the effects of compositional zonation are most striking^{2,5,12}, upward marginal flow appears unlikely even for $\Delta T \sim 0$ because of very large Le . Furthermore, geothermometric calculations based on metamorphic phase assemblages often indicate large temperature drops across pluton–countryrock contacts²⁰. Figure 2 demonstrates that even for a fast moving component, if $\Delta T > 30^\circ \text{C}$, the flow along the vertical wall will be dominated by thermal effects. Although it is possible to choose parameters such that upflow does occur for some components, compositional zonation in terms of SiO_2 is essentially precluded due to the very large Lewis number for silica in magma. It, therefore, seems that, unlike the laboratory experiments in which counter-current flow leads to a box-filling compositional stratification, the silica-rich zoned tops of magma reservoirs have a different origin.

In the case of an isothermal chamber ($\Gamma = 0$), a buoyant plume could exist along the sidewalls for components with $Le \leq 10^6$ (see Fig. 1c). An important question is whether ascending marginal flow is sufficient to generate compositionally evolved

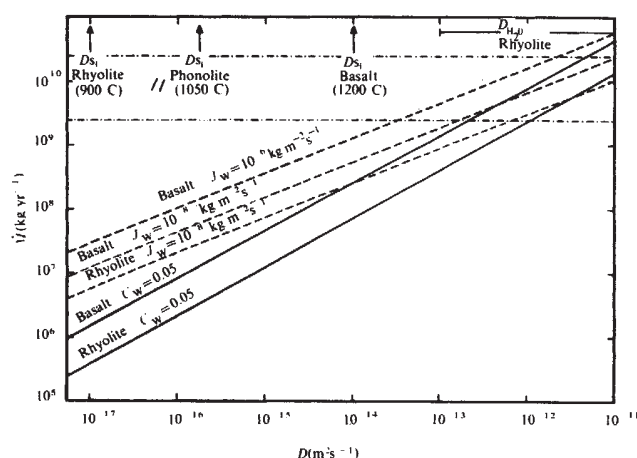


Fig. 3 Box-filling mechanism for growth of a compositionally stratified cap in a crustal magma chamber. The model is applicable to the isothermal-constant viscosity magma chamber (see Fig. 1c). A cubical magma reservoir of volume W^2L is assumed.

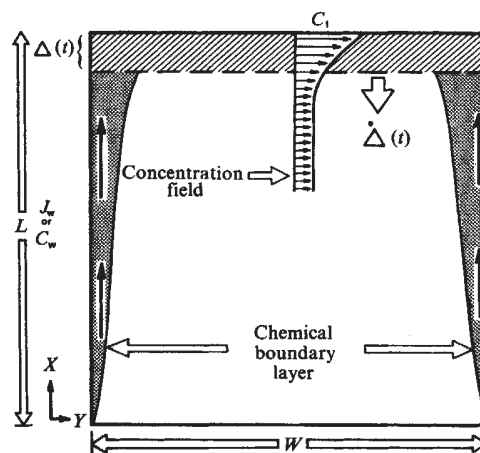


Fig. 4 Accumulation rate for evolved magma at the top of a chamber as a function of mass diffusivity of the buoyancy-generating species. Magma properties as in Fig. 2. Additional parameters are $\Delta C = 0.05$, $\nu = 3.8 \times 10^{-2} \text{ m}^2 \text{ s}^{-1}$ (basalt) and $10 \text{ m}^2 \text{ s}^{-1}$ (rhyolite). $L = W = 10 \text{ km}$. The horizontal dot-dashed lines represent inferred rate of evolved magma accumulation. The estimated range of roofward accumulation rates is based on detailed volcanological, petrological and geochronological studies. Dashed curves give M for rhyolitic and basaltic chambers subject to a constant flux of injected low-density component along the vertical boundary. Solid curves are for the constant wall composition case with $\Delta C = 0.05$.

magma at the top of the chamber equal to the inferred rate of about $10^{-2.5 \pm 0.5} \text{ km}^3 \text{ yr}^{-1}$ (refs 2, 4).

To answer this question, numerical solution of the appropriate boundary layer equations have been obtained. Details are given in ref. 21, so here we just outline the procedure and quote the results. For the isothermal problem, the relevant partial differential equations include conservation of mass, momentum and concentration of the buoyancy-inducing component. One may use similarity variables to simplify the form of these equations. Examination of the resulting set of ordinary differential equations for either the constant wall composition ($C_w = \text{constant}$) or constant wall flux ($j_w = \text{constant}$) boundary condition reveals that the Schmidt number ($Sc \equiv \nu/D$) is the only parameter of the problem. Because Sc is very large ($10^{10} < Sc < 10^{20}$), a natural method of solution is to seek an asymptotic solution valid for large Sc . Physically, this corresponds to dividing the marginal boundary layer into a thin inner chemical boundary layer embedded within a thicker momentum boundary layer. Following ref. 22, the method of matched asymptotics is

utilized to determine the velocity and concentration throughout the fluid. Solutions have been obtained for both constant wall composition and constant wall mass flux boundary conditions. The latter condition might be met in nature if low-density silicic melt is generated by partial fusion along the wall of the chamber. If this is to occur, however, large amounts of heat must be supplied to the chamber to offset the dissipated heat and the energy consumed in partial melting. A possible magmatic source of the necessary heat would be the repeated injection of primitive magma into the roots of the chamber^{5,8,23,24}. The former condition ($C_w = \text{const}$) could be attained if, for example, hydrous country rocks buffer the concentration of H_2O along the margins of the chamber¹.

The growth of a stratified layer at the top of a chamber is given by the rate at which buoyant melt crosses the plane $x = L - \Delta(t)$ (see Fig. 3). In Fig. 4, the rates of accumulation of evolved magma for various bulk compositions are plotted against mass diffusivity. The range of mass accumulation rates inferred from natural systems is also plotted. Figure 4 suggests that rates of marginal upwelling are too small by several orders of magnitude to explain gradients in SiO_2 as found in pyroclastic deposits. For H_2O , vertical stratification by the buoyant upwelling mechanism appears marginally possible. Additional calculations in which the variations of viscosity and $D_{\text{H}_2\text{O}}$ with H_2O concentration are explicitly considered are in progress.

The critical thermal Rayleigh number for the onset of turbulence is a function of the fluid Prandtl number. Because of the lower Prandtl number, turbulent convection is more probable in basaltic, as opposed to rhyolitic, chambers. Within rheological and thermal boundary layers in non-isothermal chambers however, turbulence would be suppressed. In an isothermal chamber, turbulent boundary layers might develop in a basaltic chamber. Modification of results found in ref. 27 leads to an approximate expression for the mass flow ($\dot{M}$) in a chamber characterized by turbulent boundary layers. The magnitude of $\dot{M}$ is not very different than that in the laminar flow case. In addition, the effects of entrainment and mixing²⁸ are greater in the case of turbulence and so the composition of evolved melt delivered to the chamber roof would be less silicic.

In magma chambers, where thermal and chemical buoyancy effects are present, marginal velocities are dominated by downwelling effects for components with $Le > 10^6$. Rapidly diffusing components such as H_2O in low-viscosity, basaltic reservoirs may show countercurrent flow. Upflow is maximized in isothermal chambers; however, for species other than highly mobile ones ($D > 10^{-11} \text{ m}^2 \text{ s}^{-1}$) the upward mass flow rate through the chemical boundary layer is small compared with the inferred rate of roofward magma accumulation. In an isothermal chamber, the upflow mechanism is marginally feasible in generating roofward accumulations of magma zoned in H_2O ; vertical zonation with respect to silica appears to be precluded, however.

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Cause and implications of rock varnish microchemical laminations

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Rock varnish is a dark coating composed mostly of clays and manganese and iron oxides¹ that accretes on rock surfaces in all terrestrial environments². Perry and Adams³ first observed micron-scale manganese-rich and manganese-poor (relatively iron-rich) layers in varnish and proposed that these may be related to unspecified environmental changes. Electron microprobe analyses reported here suggest that Mn:Fe ratios vary systematically with depth in varnishes on K/Ar-dated volcanic rocks from the Coso and Cima volcanic fields, eastern California, and on talus flatirons in the Negev Desert, Israel. These microchemical laminations probably reflect past fluctuations in the level of aeolian alkalinity, and possibly climatic change. This new indicator of terrestrial environmental change may be of importance to research on geomorphology, archaeology, palaeoclimatology, and Quaternary studies in arid environments.

Several lines of evidence support the premise that alternating manganese-poor and manganese-rich laminae in varnish on subaerial exposed rock surfaces are linked to the alkalinity of the aeolian environment. (1) The manganese-oxidizing microorganisms that are probably responsible for manganese enhancement in varnish^{2,4} are inhibited by alkalinity^{4–6}. (2) If manganese concentration occurs by a physicochemical fractionation process^{7,8}, where manganese is mobilized from ambient material and fixed in varnish under slight pH–Eh fluctuations and iron is not, a lack of periodic fluctuations to slightly more acidic conditions in a geochemical regime that is highly alkaline could also promote a varnish poor in manganese. (3) Except for acid springs and acid mine-drainages where iron is fixed by chemolithotrophic bacteria⁹, orange (manganese-poor) varnishes only occur in deserts where rock surfaces are in constant, direct contact with alkaline material. In contrast, manganese-rich (black) varnishes have been observed in all terrestrial weathering environments that are not acidic enough to mobilize manganese from surficial coatings². (4) Contemporary manganese-rich varnishes have near-neutral pH values, and manganese-poor varnishes have alkaline pH levels¹⁰.

However, several potential sources of error may confuse this assumed relation between varnish microchemical laminations and regional alkalinity levels. (1) Dominant local influences could interfere with a regional signal. For instance, accumulation of organic material from adjacent plants or airborne fallout from a nearby deflation surface could inhibit manganese oxidation by microorganisms. (2) Microlaminations are usually discontinuous from microdepression to microridge (on a millimetre to centimetre scale), and they can sometimes be discontinuous within the same microdepression. These discontinuities may be caused by differential rates of varnish accumulation, differential compression of subsurface varnish, anomalous alkaline detritus (see Fig. 2b in ref. 10), and/or localized chemical or mechanical

erosion of varnish. (3) Manganese-poor layers could represent periods when manganese is leached, leaving an iron-rich lag^{2,8}. However, Perry and Adams³ and Elvidge and Iverson⁷ feel that laminations are primary depositional features, and an erosional cause has also been disputed². First, the surface layers of varnish in less arid environments (more chemically erosive to manganese) are manganese-rich, whereas varnishes with iron-

rich surface layers are found in alkaline, hyper-arid environments. Second, although microenvironmental variations between microdepressions and microridges can cause discontinuities in laminations, it is less likely that a major climatic change would be as discriminating. (4) In rock crevices in arid environments, orange (manganese-poor) varnish often develops in direct contact with alkaline desert dust. With spalling, this

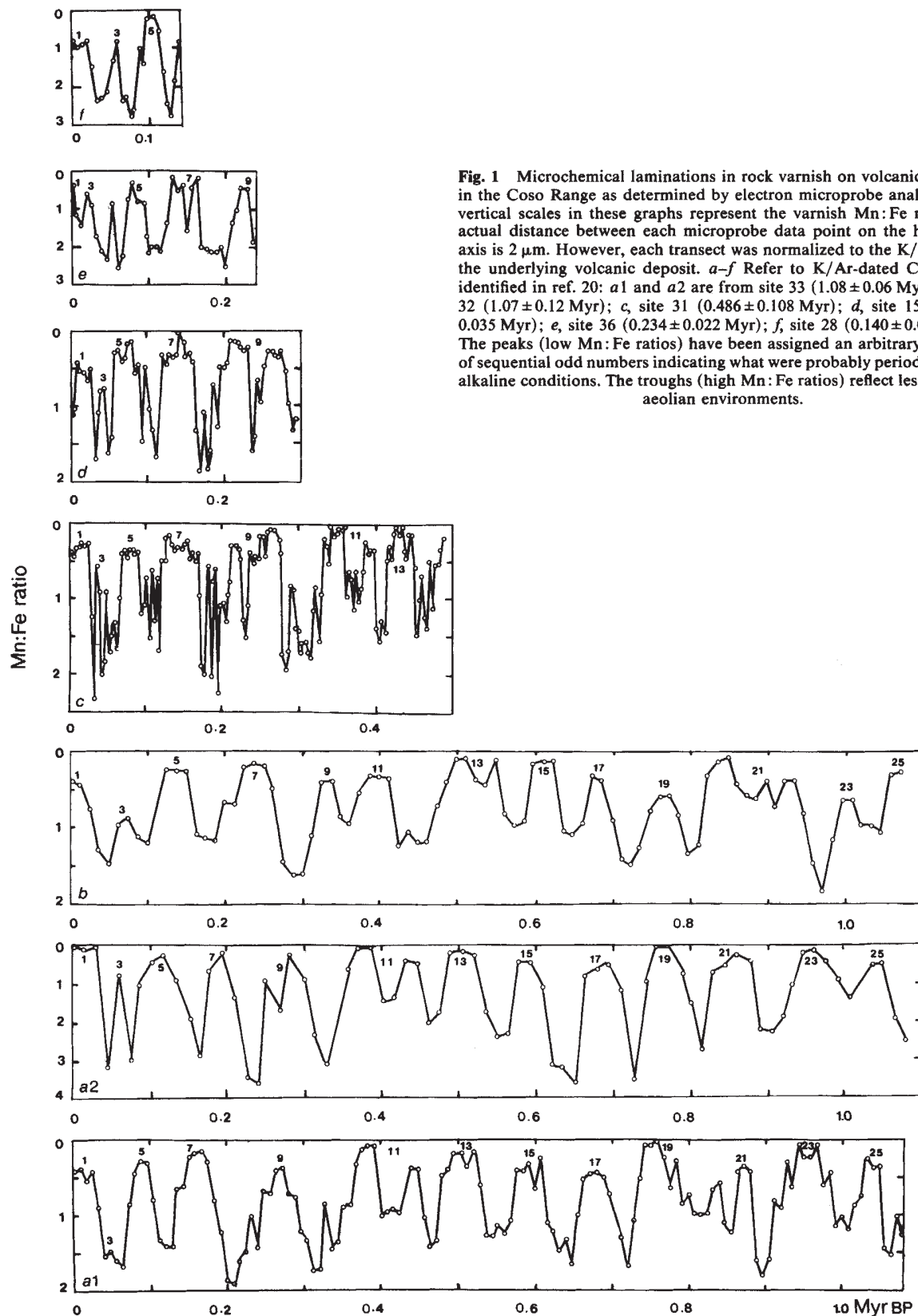


Fig. 1 Microchemical laminations in rock varnish on volcanic deposits in the Coso Range as determined by electron microprobe analyses. The vertical scales in these graphs represent the varnish Mn:Fe ratio. The actual distance between each microprobe data point on the horizontal axis is 2 μ m. However, each transect was normalized to the K/Ar-age of the underlying volcanic deposit. *a-f* Refer to K/Ar-dated Coso sites, identified in ref. 20: *a1* and *a2* are from site 33 (1.08 ± 0.06 Myr); *b*, site 32 (1.07 ± 0.12 Myr); *c*, site 31 (0.486 ± 0.108 Myr); *d*, site 15 (0.293 ± 0.035 Myr); *e*, site 36 (0.234 ± 0.022 Myr); *f*, site 28 (0.140 ± 0.089 Myr). The peaks (low Mn:Fe ratios) have been assigned an arbitrary notation of sequential odd numbers indicating what were probably periods of more alkaline conditions. The troughs (high Mn:Fe ratios) reflect less-alkaline aeolian environments.

crack varnish is exposed to less-alkaline, subaerial conditions and subsequently receives a superposed layer of black varnish enriched in manganese. Thus a basal sequence of manganese-poor under manganese-rich varnish may not reflect fluctuations in the alkalinity of aeolian fallout. (5) Some varnishes have microprobe Mn:Fe profiles that reveal no laminations. This may be due to improper thin-section preparation, varnish accretion rates that are too slow to record these environmental changes, or environments that do not experience significant fluctuations in alkalinity.

To assess the consistency and significance of Mn:Fe microchemical laminations, varnishes were sampled from the surfaces of 11 K/Ar-dated rhyolite domes and south to south-west facing basalt flows in the Coso Range, eastern California. Because the focus of this study is on the sensitivity of varnish to regional fluctuations in levels of aeolian alkalinity, varnish samples were only collected from the tops of rock exposures at least 1 m above adjacent soil surfaces.

Figure 1 presents electron microprobe transects from the varnish surface to the underlying rock, each representative of multiple microprobe transects of the varnish analysed from six of the volcanic flows. Varnishes of five other volcanic deposits in the Coso Range were analysed and not reported here, but microprobe analyses reveal very similar results (R.I.D., in preparation). Successive microprobe analyses were taken at 2- μ m intervals. For ease of comparison, varnish depths were normalized to the K/Ar-age of the underlying deposits. Note that the time intervals in Fig. 1 are only approximations, because: (1) although the rate of varnish accretion was assumed to be constant for the construction of Fig. 1, this rate probably varies; (2) different time lags may occur between exposure of a surface to the subaerial environment and the onset of varnishing; (3) significant age-uncertainties are assigned to some of the K/Ar-dated volcanic flows, and (4) compression of older varnish probably occurs after burial.

Multiple microprobe transects were made into varnishes on several rocks from each of the 11 Coso flows. *a1* and *a2* in Fig. 1 exemplify variations that can be found in transects from adjacent rocks in the same volcanic flow. The most consistent results occurred in 1–3 mm-wide microdepressions collected from sloping surfaces. The average rate of varnish accretion in these small hollows is probably rapid enough to record major fluctuations in regional levels of alkaline aerosols, and these microdepressions are probably too small to generate their own microenvironment. About one-third of the transects had discontinuous microlaminations or profiles with few or no variations

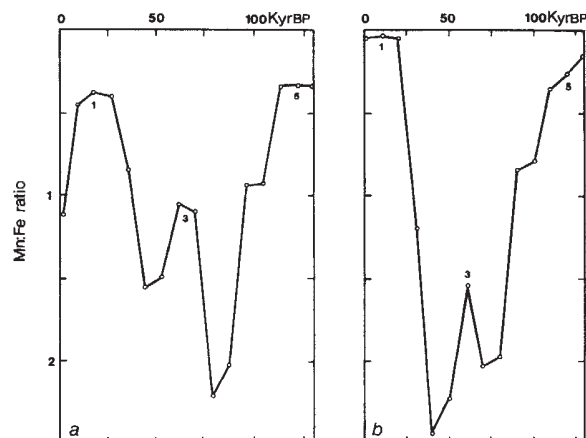


Fig. 2 Microchemical laminations of varnish on Cima basalt flow i_2 (K/Ar-age of 0.13 ± 0.06 Myr)¹¹. *a* and *b* are transects from two separate rock exposures.

in Mn:Fe ratios. The remainder are continuous and fairly consistent, including the microchemical laminations presented in Fig. 1.

Figure 2 presents microprobe transects from rock varnish on two separate rock outcrops on a 130,000 $\pm$ 60,000 BP K/Ar-dated basalt flow in the Cima volcanic field of eastern California¹¹. Like the Coso Range, varnish on the Cima basalt flow was collected from constructional surfaces. The sequence of high and low Mn:Fe ratios in the Cima varnish is remarkably similar to the sequence of Mn:Fe ratios from the Coso Range over the same approximate time period.

Figure 3 illustrates the microlaminations of varnishes on talus flatirons in the Negev Desert of Israel. Gerson¹² proposed a climatic-geomorphic model for the development of sequences of inset flatirons, where talus is built up in lengthy, more humid periods and eroded during arid phases of long duration. According to Gerson's model the varnishes on the oldest of three talus flatirons should have experienced three major arid periods separated by two long humid periods. This is indeed reflected in microprobe transects of varnish on the oldest flatiron at two localities in the Negev Desert (Fig. 3). The low Mn:Fe ratio of the basal layer may represent varnishing during an arid-alkaline phase after the humid period when the talus was deposited. Above this iron-rich band are two cycles of manganese-rich and

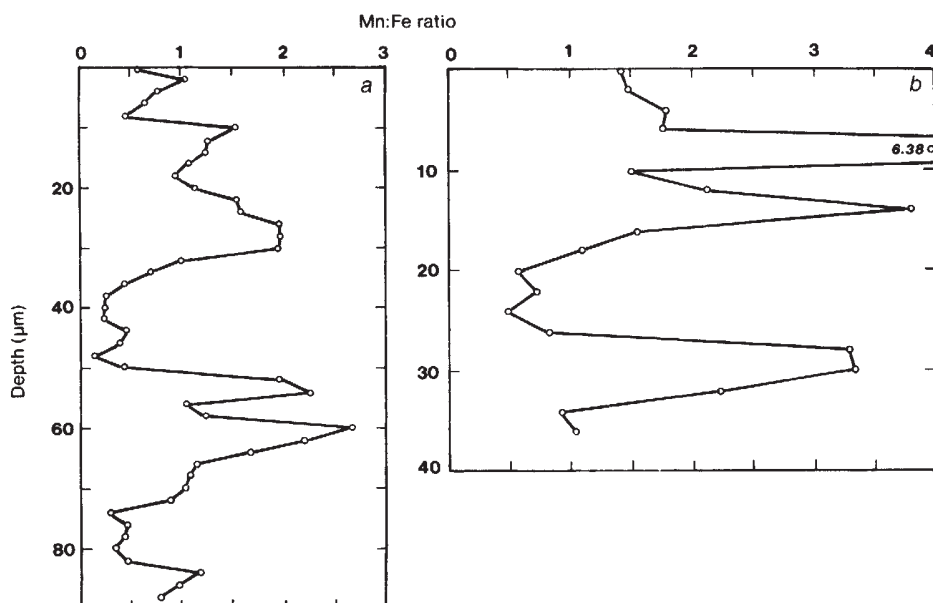


Fig. 3 Microprobe profiles of varnish collected from the oldest flatiron in sequences of inset talus flatirons in the Negev Desert, Israel. *a*, Representative transect of varnish on syenite in the Timna Valley, southern Negev. More humid periods are interpreted as occurring from ~8–34 μ m and from ~50–70 μ m. *b*, Representative transect of varnish on chert near Paran in the Arava Valley, central Negev. More humid phases are likely from ~6–16 μ m and from ~26–34 μ m. Because no absolute age-estimates are available, the depth from the surface to the underlying rock is plotted against the Mn:Fe ratio.

manganese-poor laminations, corresponding with the development of the two younger talus flatirons at each site. Microprobe analyses of varnishes on the younger flatirons are also consistent with Gerson's model (R.I.D., in preparation).

The consistency of these preliminary results warrant some tentative interpretations. The sites reported here are adjacent to basins that have alternated between pluvial lakes and saline playas during the Quaternary¹³⁻¹⁵. Similarly, when these lakes were present, the vegetative cover was probably more extensive¹⁶⁻¹⁸. During presumably warmer-drier climates, saline playas developed and the vegetation cover over desert soils decreased, providing a more abundant source of alkaline aerosols¹⁹ that probably fell on neighbouring upland areas. Thus, the climate of these areas may have controlled fluctuations in the concentrations of alkaline aeolian fallout and hence influenced Mn:Fe ratios in varnishes. High ratios probably reflect less alkaline conditions, and low ratios probably indicate more alkaline conditions. The sensitivity of varnish Mn:Fe ratios to fluctuations in alkalinity appears to be of the order of 10,000–25,000 yr and is probably dependent on the rate of varnish accretion.

Note that each individual microprobe transect only reflects the history of influences imposed on that given rock. Systematic sets of intra-rock, inter-rock, and inter-site transects of microchemical laminations are required before a record of regional changes in the aeolian fallout can be reasonably interpreted. These have been obtained for the Coso Range (R.I.D., in preparation), although more extensive microprobe work is still required to determine conclusively under what conditions uniform lamination sequences develop. The offsets that do occur between the varnish profiles in Fig. 1 may result from: differences in the time required to initiate varnishing on the volcanic deposits; uncertainty in the K/Ar-ages; variable rates of varnish accretion, and differential compression, particularly with respect to the most recent peak of low Mn:Fe ratios. This most recent peak, labelled as stage 1, lacks the compression that is likely to occur in older varnish after burial. The greater relative length of stage 1 may also in part be due to the possible attenuation of this surficial layer of varnish during thin section preparation, and a faster rate of clay accumulation during the Holocene and similar environments.

Over approximately the past million years in the Coso Range, eastern California, there have been systematic fluctuations in rock varnish Mn:Fe ratios that probably reflect variations in regional aeolian alkalinity and possibly changes in climate. These rock varnish microchemical laminations are also documented in the Mojave Desert of eastern California and in the Negev Desert of Israel. However, before microchemical laminations can be interpreted reliably as a record of fluctuations in alkaline aeolian fallout, more data are needed to establish conclusively the link between Mn:Fe ratios and alkaline aeolian fallout and to assess the factors that influence inconsistencies in rock varnish microchemical laminations. This new record of terrestrial environmental change during the Quaternary is speculative, but it has considerable potential as a long-term indicator of past climates and as a tool to correlate landforms in what are at present arid lands.

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Dimethyl sulphide in a stratified coastal salt pond

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Dimethyl sulphide (DMS) is the major volatile reduced organic sulphur compound in open ocean and coastal waters, and its emission from surface water represents a major flux of biogenic reduced sulphur to the atmosphere¹⁻⁸. It is an algal natural product^{4,9-11} as well as a product of microbial decomposition of organic matter¹²⁻¹⁵. In the open ocean, the distribution of DMS is correlated with primary productivity^{3,4,6,8}. In a study of a stratified coastal salt pond reported here, DMS was the predominant volatile sulphur compound in the oxic epilimnion throughout the year. There was consistently a peak in DMS concentration just above the oxic–anoxic interface at low oxygen tensions, probably arising from a combination of physiological stress and decomposition of algal material. During winter and early spring, a second DMS peak was present in the epilimnion, probably associated with algal production. Elevated DMS concentrations did not seem to be related to strict anoxia, as the lowest concentrations were always found in the anoxic hypolimnion where H₂S (a volatile reduced inorganic sulphur compound) dominates.

Salt Pond is a shallow (5.5-m deep) eutrophic marine basin on Cape Cod, Massachusetts¹⁶, that exhibits density stratification. While aerobic processes dominate the epilimnion, the anaerobic hypolimnion generally has high concentrations of H₂S (up to ~5 mM) generated from sulphate reduction in the bottom waters and sediments. In summer, the anoxic zone rises to within 2–3 m of the pond surface and the oxic–anoxic interface becomes quite pronounced, resulting in steep geochemical gradients. During winter, the depth of the interface deepens and H₂S concentrations decrease as a result of wind-driven mixing. Occasionally the water column overturns.

DMS concentrations were measured in the water column of Salt Pond between June 1982 and July 1983. Water samples were collected along a vertical profile in the central basin using a battery-operated peristaltic pump and a weighted silicon rubber tube¹⁷ lowered sequentially to the appropriate sampling depths. Glass sample bottles (2 l) were rinsed, filled and stoppered without headspace. The water samples were returned to the laboratory within ~1 h of collection and were syringe-filtered through 0.22-µm Millipore filters to remove algal cells and poisoned with HgCl₂. HgCl₂ acted both to preserve the samples and to precipitate H₂S which, when present at high concentrations, interferes with our DMS assay. Volatile compounds were collected from the water subsamples (50–200 ml) by sparging

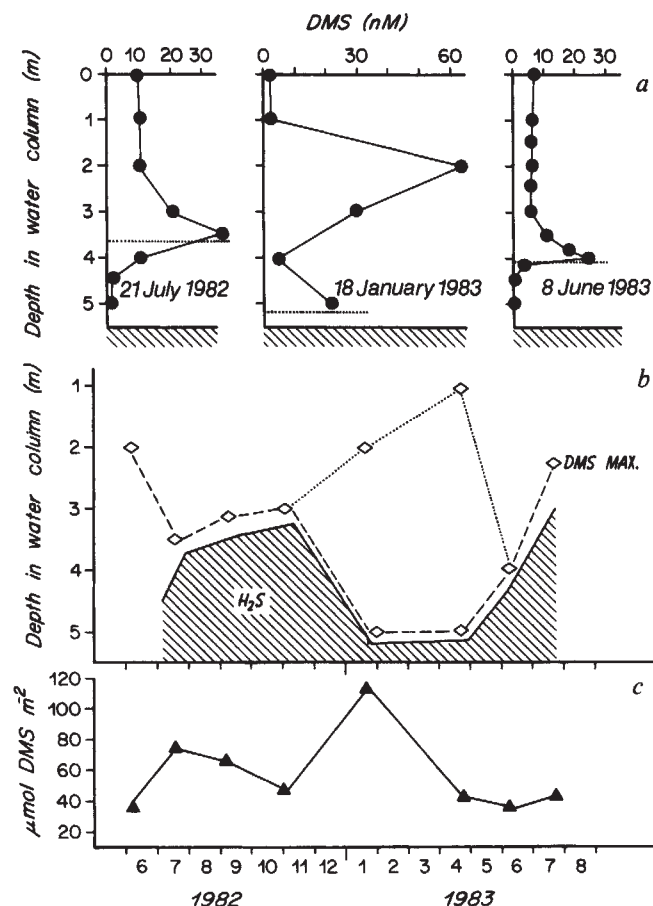


Fig. 1 DMS distributions in Salt Pond. *a*, Selected DMS (nM) depth profiles; dotted lines indicate approximate depth at which H_2S was detected. *b*, Seasonal variation of the depth of maximum concentrations of DMS and approximate depth of the O_2/H_2S interface. *c*, Seasonal DMS inventory (0–5.5 m).

for 20 min with helium at 35 °C and cryogenic trapping (liquid N_2) on glass beads. The trapped components were then cryo-focused at the head of a glass capillary gas chromatographic column (50 m $\times$ 0.3 mm i.d.; 0.5- μ m film of Pluronic 121) for subsequent separation. Sulphur-containing compounds were measured with a flame photometric detector. Thiophene was added to each sample as an internal standard before sparging. Recovery of DMS from pond water was about $85\% \pm 10\%$. All DMS concentrations have been corrected for recovery and blanks. Analysis of unfiltered and unpoisoned samples from the epilimnion gave the same DMS results as treated samples. Temperature, salinity, pH, Eh (polished platinum electrode) and pS^{2-} (ref. 18) were measured in pond water pumped through an in-line flow chamber as samples for DMS were being collected. The vertical profile of dissolved oxygen was determined by lowering a temperature- and pressure-compensated Clark-type O_2 electrode through the water column just into the anoxic zone. Oxygen measurements have been corrected for salinity. Suspended particulate carbon concentrations were determined by CHN analysis of particles obtained by filtration onto precombusted glass fibre filters, and pigments by acetone extraction of filtered particles (0.45- μ m Millipore filters) followed by spectrophotometric analysis (665 and 750 nm before and after acidification).

DMS was the most abundant volatile reduced organic sulphur compound in the epilimnion of Salt Pond throughout the year. DMS concentrations ranged from near its detection limit (~ 0.5 nM) up to 62 nM, with distinct peaks in concentration (Figs 1, 2). These concentrations are generally higher than surface seawater values (2–5 nM; refs 1, 3, 5, 6, 8) but lower than the $\sim 1,000$ nM found in a shallow (1.5 m) eutrophic fresh-water pond⁹. Carbonyl sulphide, methyl mercaptan, carbon disulphide and dimethyl disulphide were occasionally present, but concentrations were 2–10 times lower than those of DMS.

The distribution of DMS in the water column followed a seasonal cycle in apparent association with two factors: (1) the depth of the O_2-H_2S interface during all seasons; and (2) phytoplankton in the epilimnion during winter and spring. Throughout the year, a DMS concentration peak was found

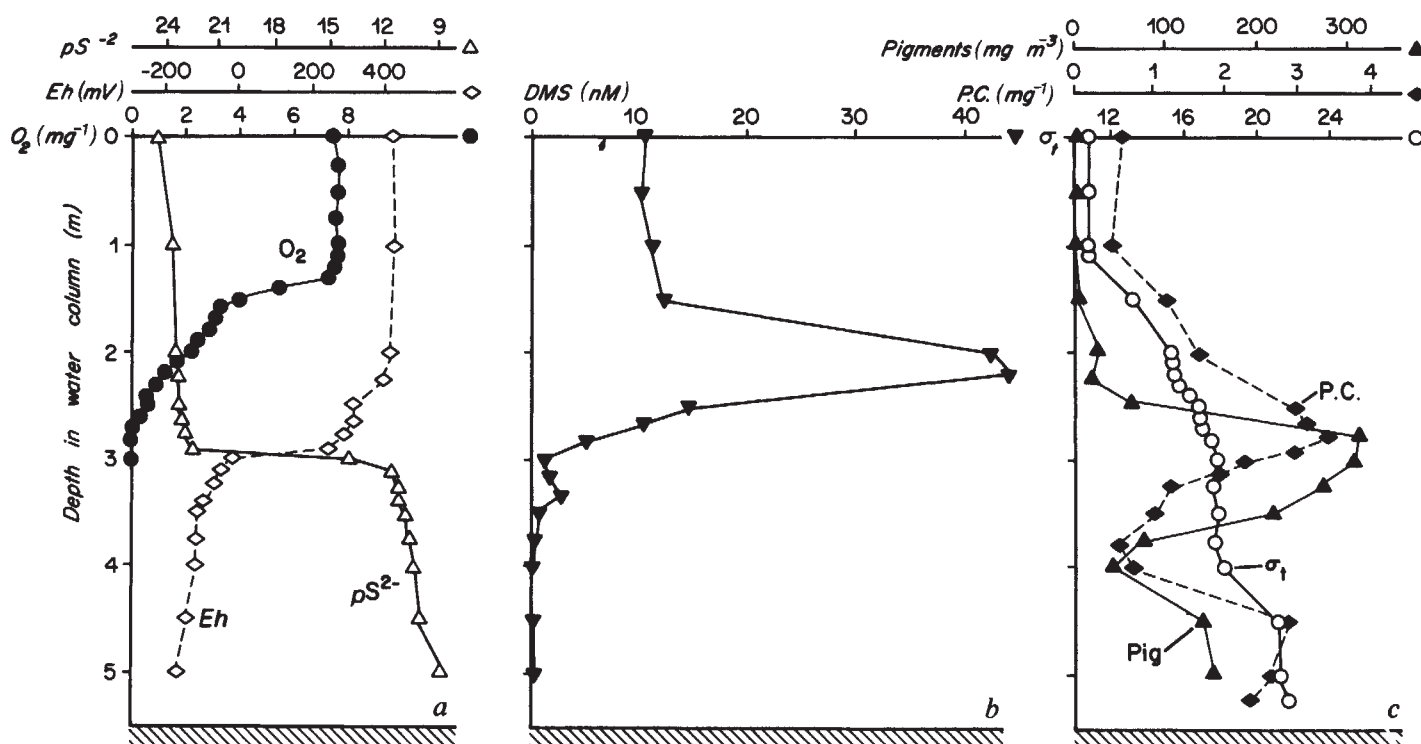


Fig. 2 Vertical profiles of DMS and geochemical parameters in Salt Pond, 26 July 1983. *a*, Dissolved oxygen, Eh, pS^{2-} . *b*, DMS (nM). *c*, Density (σ_t), suspended particulate carbon (P.C.), total pigments.

immediately above the oxic-anoxic boundary (Fig. 1a, b). During seasonal excursions of the interface depth (Fig. 1b), the DMS peak shifted accordingly. A detailed examination of water column chemistry (Fig. 2) shows that the DMS peak is actually in the zone in which dissolved oxygen concentrations are decreasing, but above which H_2S appears. The DMS peak also lies above the peaks in particulate carbon and pigments, both of which straddle the pS^{2-} and Eh breaks and probably correspond to high rates of bacterial activity. The cause of the maximum DMS concentration is unclear. It may result from the decomposition of phytoplankton or other detritus at the interface, or from the effect of low oxygen tensions creating a physiological stress on phytoplankton near the interface, or both. Decomposition of algal material releases DMS in both aerobic^{9,15} and anaerobic^{13,14} environments. The most likely precursor compound is the algal osmoticum, dimethylsulphoniopropionate (DMSP; dimethylpropiothetin)¹⁹⁻²⁴ which releases DMS on enzymatic cleavage. DMS formation does not seem to be associated with the sulphide produced from sulphate reduction since the maximum DMS concentrations were always in the oxic zone of the water column. At all times, DMS levels below the O_2 - H_2S interface were near or below the detection limit, consistent with anaerobic metabolism of DMS²⁵.

During winter and early spring, when the O_2 - H_2S interface was near the bottom of the pond, a second DMS peak was observed in the upper epilimnion. This additional peak we attribute to release by phytoplankton. DMS is released by axenic cultures of a variety of phytoplankton^{4,9-11}, and a close correspondence between DMS and chlorophyll *a* profiles has been noted in the open oceans^{3,4,6,8}. We do not have chlorophyll *a* data for Salt Pond (the pigment profile in Fig. 2 probably represents partially degraded bacterial pigments), but spring blooms commonly occur in the pond (C. D. Taylor, personal communication). The release of DMS by phytoplankton is species specific^{4,9-11} and although DMS and phytoplankton concentrations often coincide, major blooms in Salt Pond during April and July 1983 (S. Lohrenz, personal communication) did not produce concurrent DMS peaks in the epilimnion.

An estimate of the seasonal DMS inventory in the Salt Pond water column was made by integrating the DMS concentration profiles over the 5.5-m depth of the pond. The resulting inventory ranges from 30 to 95 $\mu\text{mol DMS m}^{-2}$ (Fig. 1) and was highest in January 1983, when the highest DMS concentration (62 nM) was observed. Despite major differences in both the depth distribution and the apparent sources of DMS during the study period, the total amount of DMS in the pond did not fluctuate greatly. To maintain a relatively constant DMS inventory in the pond, there must be a balance between the rate of production and the rate of removal, either by exchange to the atmosphere, by DMS metabolism at the O_2 - H_2S interface, or by oxidation of DMS in the epilimnion. The relative constancy of DMS in the pond contrasts markedly with the other major volatile reduced (inorganic) sulphur compound in the water column, H_2S . Hydrogen sulphide was very abundant in the anoxic bottom waters during summer stratification, but was greatly depleted in winter. However, throughout the study, the total H_2S per m^2 was several orders of magnitude greater than DMS.

Our results show that DMS is the major volatile reduced organic sulphur compound in Salt Pond. Distributions of DMS vary seasonally in response to the changing physical, chemical, and biological character of the pond's water column. Two sources of DMS seem to be important. We observed elevated DMS concentrations in association with some algal blooms, as has been previously reported. However, it seems that on an annual basis, most of the DMS was produced and/or preserved in low oxygen conditions just above the oxic-anoxic interface. This may be an important mechanism for DMS formation in marine systems where oxygen becomes depleted.

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Amino acid epimerization implies rapid sedimentation rates in Arctic Ocean cores

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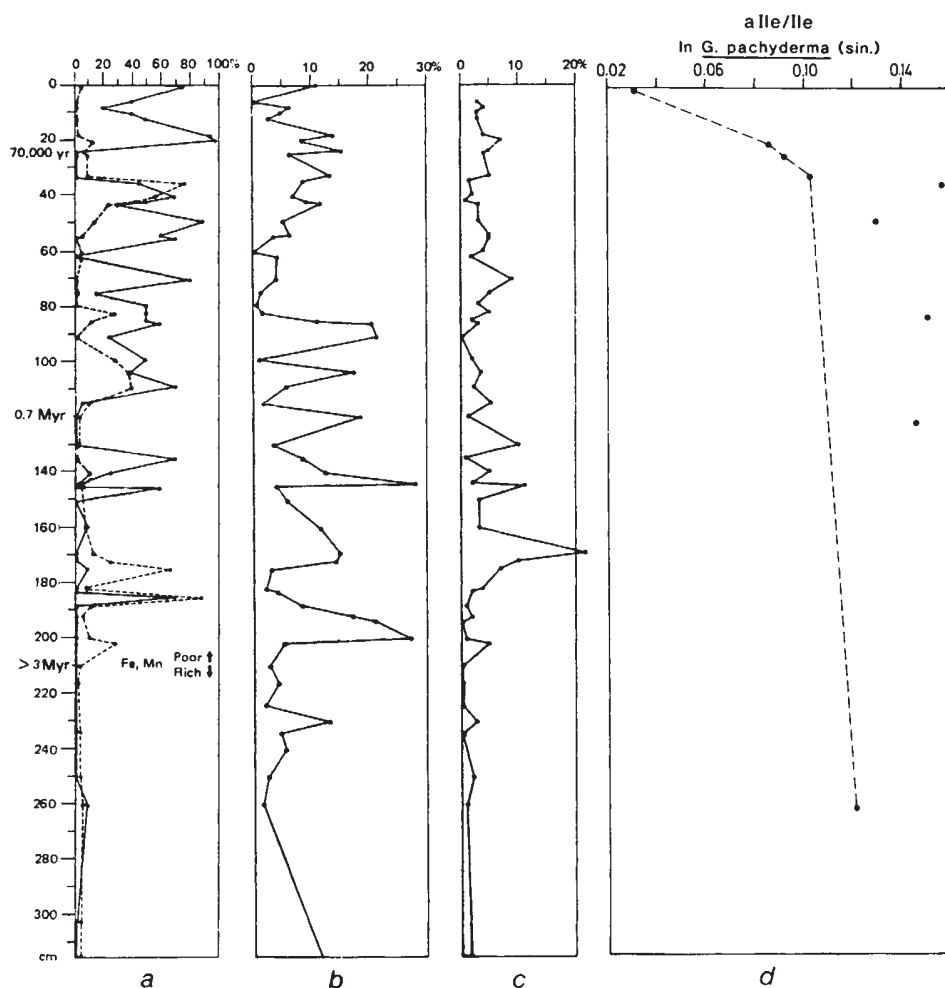
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The palaeoceanography of the Arctic Ocean is less well known than any other ocean basin, due to difficulties in obtaining cores and in providing a secure chronological framework for those cores that have been raised. Most recent investigators have suggested that low sedimentation rates (0.05-0.1 cm kyr⁻¹) have characterized the deep basins over the past 5 Myr (refs 1,2) despite the glacial-marine character of the sediment and proximity to major centres of shelf glaciation. These calculations have been primarily based on the down-core pattern in the inclination of magnetic minerals, supported by uranium-series, ¹⁴C and micropalaeontological evidence. Here we analyse amino acid diagnosis in foraminifera from two gravity cores raised from the floor of the Arctic Ocean, our results suggest that these cores span <200 kyr., conflicting with the earlier estimate of 3 Myr based on palaeomagnetic data. The chronology of other Arctic Ocean cores and previous palaeoenvironmental interpretations need re-evaluation.

The present study is mainly based on samples of planktonic foraminifera extracted from the 260-cm long Arctic Ocean core T3-67-11 (79°34.9' N 172°39' W) raised from a depth of 2,810 m. Because of the possibility that planktonic foraminifera are reworked from shelf areas, we have also analysed three samples of benthic deep-water foraminifera. Two lots of samples were extracted from core T3-67-3 as core T3-67-11 did not contain enough benthic individuals. Core T3-67-3 was raised from a depth of 2,285 m (79°11' N 175°09' W) and was 380 cm long. Palaeomagnetic data, sedimentology, and micropalaeontological results from these cores have been published elsewhere^{1,3-5}.

Fig. 1 Amino acid data plotted against some sedimentological data from the Arctic Ocean core T3-67-11 (from ref. 1). *a*, Solid line, microfauna (left) to clastics (right) in the coarse fraction ($> 62 \mu\text{m}$). Dashed line, percentage of *Globigerina quinqueloba* complex of the total planktonic foraminifera population. *b*, Percentage of coarse fraction ($> 62 \mu\text{m}$). *c*, Percentage of benthic foraminifera of the total foraminiferal fauna. *d*, Alle/Ile ratios in the total fraction in *Globoquadrina pachyderma* (sin.).



Amino acid diagenesis of the indigenous protein preserved in fossils is a useful stratigraphical and geochronological tool for material of Quaternary to late Tertiary age^{6,7}. Over the past decade, the racemization and epimerization reactions in carbonate fossils have received much attention⁸⁻¹³. The epimerization reaction between the protein amino acid L-isoleucine (Ile) and the nonprotein amino acid D-alloisoleucine (alle) is utilized in the present study. The extent of this reaction is expressed as ratio of alle/Ile, which increases with time from close to zero in modern samples to an equilibrium value of ~ 1.3 . The reaction rate is temperature dependent (activation energy $\sim 28 \text{ kcal mol}^{-1}$) and at 0°C , 10^6 – 10^7 yr should be required to attain equilibrium. Material from the deeper parts of the world's oceans should be well suited for this method because of the relatively stable temperature conditions in these areas in both time and space. The isoleucine epimerization reaction has been used in studies of deep-sea cores both on bulk sediment¹⁴, and on samples of planktonic foraminifera¹⁵⁻¹⁸. As in other carbonate fossils, the epimerization reaction rate varies between foraminifera taxa^{13,17}, and therefore, only monospecific samples are used.

For this study, analyses were performed on samples of the planktonic foraminifer *Globoquadrina pachyderma* (sin.) from nine different levels in core T3-67-11. In addition, the extent of isoleucine epimerization in the benthic species *Cibicides wuellerstorfi* was measured in one sample from T3-67-11 and two samples from T3-67-3. Each preparation consisted of 40–80 individual tests following the procedure outlined by Miller *et al.*¹³, and was analysed on an HPLC ion-exchange amino acid analyser. Only the total amino acid fraction (free plus peptide-bound) was analysed; the alle/Ile ratios were determined by measuring peak heights (Table 1).

Figure 1 shows the ratios from core T3-67-11 plotted against depth along with some sedimentological parameters and the palaeomagnetic-based time scale. The four samples from the upper 33 cm in core T3-67-11 show a steady down-core increase in alle/Ile values from 0.033 to 0.104. However, the next five samples vary between 0.157 and 0.121. The lowest ratio in this latter group came from the deepest sample analysed in the core (260 cm depth). The most reasonable explanation for the erratic variations in alle/Ile ratios in the lower part of core T3-67-11, is redeposition. The amount of coarse material and presence of shallow-water benthic foraminifera of the genus *Elphidium* throughout most of the core indicates that ice-rafting from shelf areas has occurred¹. Accordingly, it is reasonable to assume that some of the planktonic tests may also be reworked. When analysing samples consisting of 40–80 tests, the resulting alle/Ile ratio represents a weighted mean of the extent of epimerization in all tests. Analyses that include older redeposited foraminifera will always give ratios that are too high. The more consistent pattern of the ratios above 33 cm might suggest that the relative proportion of redeposited foraminifera in this part of the core is lower than below 33 cm. Because redeposition is likely to have occurred throughout the core, the measured alle/Ile ratios should be regarded as maximum values when evaluating the age of the sediment.

Age estimates based on the amino acid data can be made: (1) by applying equations based on kinetic studies and assuming a reasonable temperature history for the sample; and (2) by comparing the alle/Ile values with ratios obtained on similar species from well-dated cores from other deep ocean basins.

Few data have been published concerning the relationship between the reaction rate of isoleucine epimerization and temperature. Miller *et al.*¹³, have determined this relationship for

Table 1 Alle/Ile ratios in *Globoquadrina pachyderma* and *Cibicides wuellerstorfi* (total fraction) from Arctic Ocean cores T3-67-11 and T3-67-3

Lab. no.	Core no.	Depth in core (cm)	Alle/Ile
AAL 1940	T3-67-11	Top	0.033
AAL 2078	T3-67-11	20 cm	0.085
AAL 2077	T3-67-11	25 cm	0.091
AAL 1938	T3-67-11	33 cm	0.104
AAL 1937	T3-67-11	35 cm	0.157
AAL 2079	T3-67-11	49 cm	0.130
AAL 1939	T3-67-11	86 cm	0.152
AAL 2076	T3-67-11	120 cm	0.145
AAL 1936	T3-67-11	260 cm	0.121
BAL 75	T3-67-11	Top	0.019
BAL 76	T3-67-3	98-101 cm	0.105
BAL 74	T3-67-3	321 cm	0.140

the benthic species *Cibicides lobatulus*. Assuming that the alle/Ile ratio from the 260-cm level (0.121) is closest to the *in situ* ratio below 35 cm in core T3-67-11 and that the temperature has always been -0.5°C (the present temperature), the equation for *C. lobatulus* in ref. 13 gives an age of 188,000 yr BP for the lower part of the core. The temperature in the deeper part of the Arctic Ocean cannot have been much lower than this. If we assume a temperature of 3°C , then the *C. lobatulus* equation gives an age of 117,800 y BP. Note that these estimates do not take into consideration the species-dependent differences in the epimerization rate between *C. lobatulus* and *G. pachyderma* and that the measured alle/Ile ratio may well be too high due to reworking. Nevertheless, the results of these calculations differ dramatically from the >3 Myr age inferred for the same level of the core from palaeomagnetic data.

Alle/Ile ratios were determined on *G. pachyderma* (sin.) from the well-dated North Atlantic core K-708-7 for comparison with the data from the Arctic Ocean. Samples from 140 and 755 cm, corresponding to ages of 50 and 325 kyr, were analysed. The measured alle/Ile ratios in these samples are 0.078 and 0.21 respectively. Although the present bottom-water temperatures over core K-708-7 in the North Atlantic are $2-3^{\circ}\text{C}$ higher than those in the Arctic Ocean, this difference was much less during glacial episodes¹⁹ when bottom water formation in the Norwegian Sea was limited²⁰. Assuming even the highest possible temperature difference, these data suggest that the 0.121 alle/Ile ratio from the bottom of core T3-67-11 was established less than 300 kyr ago. King and Neville¹⁷ have reported down-core increases in alle/Ile-ratios in the planktonic species *Globigerinoides sacculifer* and *Globorotalia tumida* from the well-dated Pacific core V28-238. The maximum temperature difference between their core site and the Arctic Ocean is 2°C . Plotting our 0.121 ratio in *G. pachyderma* on their figure gives an age of ~ 70 kyr on the *G. tumida* curve and ~ 120 kyr on the *G. sacculifer* curve, similar to the ages derived from the North Atlantic calibration.

As a secondary check on the planktonic data we also determined the alle/Ile ratios in the benthic foraminifer *Cibicides wuellerstorfi*, a deep-water species less likely to have been reworked by shelf glaciation. The core-top sample from T3-67-11 (BAL-75, Table 1) gave essentially a modern ratio, confirming that no significant reworking is occurring at present. Samples from core T3-67-3 were analysed from the 98 to 101 cm level (alle/Ile = 0.105) and the 321 cm level (alle/Ile = 0.140). Using the Miller and others¹³ equation and assuming a temperature of -0.5°C , these ratios give ages of 160 and 220 kyr, respectively. The Bruhnes-Matuyama boundary (700 kyr) is placed at 240 cm depth in this core¹.

We conclude from the amino-acid data that the deepest portions of core T3-67-11 (260 cm) were probably deposited more recently than 200 kyr ago and that much of the fauna in the lower part of the core is reworked. The amino-acid derived ages are dramatically younger than the palaeomagnetic interpretation

(3 Myr for the same core level), and require a re-evaluation of the sources of error in the magnetic signal.

Previous studies of Arctic Ocean sediment cores have used the down-core variations in magnetic signature to establish a palaeomagnetic time frame. Because the magnetic signatures alone do not contain a primary age signal, it is necessary to correlate the observed magnetic patterns to the established late Cenozoic reversal time scale. Reliable magnetostratigraphical correlation requires unambiguous identification of reversal boundaries and consistent orientation within inferred polarity intervals. Although declinations in high-latitude cores are likely to be obscure, inclination data should be adequate for defining reversal boundaries. Clark *et al.*² note that the change from normal to reversed inclinations occurs gradationally over a 15-40-cm interval. Late Cenozoic geomagnetic reversals are thought to occur in <10 kyr (ref. 20). Based on the proposed sedimentation rates, this should occur over <3 cm of core. The broader transition period reported by Clark *et al.*² conflicts with the expected transition interval and may reflect a more rapid sedimentation rate. The published data on the magnetic signature measured within polarity intervals for the Arctic Ocean cores show considerable scatter and this noise is unexplained. It may be due to the influence of large individual pebbles scattered throughout the cores or to postdepositional processes which may both alter and obscure recorded directions of the geomagnetic field²¹. There are many difficulties in obtaining reliable palaeomagnetic orientations in stony glacial-marine sediment. Most of the published Arctic Ocean palaeomagnetic results are inadequately documented; no data are presented for directional distributions or the behaviour of sample signal during demagnetization procedures. Detailed demagnetization treatment of some Barents Sea cores (R.L., in preparation) shows that even a treatment to 60 mT is sometimes not capable of removing magnetic overprints. We conclude that the published palaeomagnetic information from Arctic Ocean cores is not sufficiently consistent to exclude alternative interpretations of the core ages and sedimentation rates.

The bottom water masses in the Arctic Ocean and the Norwegian-Greenland Sea are not separated by any shallow sills. They have similar physical characteristics and are mainly formed in the area between Jan Mayen and Spitsbergen by cooling and sinking of high-salinity Atlantic surface water. Benthic organisms in the deeper parts of these oceans should react in a similar manner to a change in the mode and mechanism of deep-water formation.

The lower portions of many of the cores from the Arctic Ocean have a poor benthic foraminifera fauna, some of them dominated by the genus *Elphidium*^{1,5,6}. Often typical benthic deep-water species are found only in the upper parts of the cores. The *Elphidium* species (mostly *Elphidium excavatum*) are supposed to have been derived from shelf areas by ice-rafting¹.

A similar stratigraphy has been recorded in cores raised from the deeper part of the Norwegian and Greenland Seas²²⁻²⁴. Here a zone almost barren of benthic foraminifera and dominated by *Elphidium* is found in sediments deposited during oxygen-isotope stages 4-2. The migration of deep-water species into the Norwegian Sea close to 13 kyr ago is explained by a new influx of better oxygenated deepwater into the Norwegian Sea after a long period of isolation from the North Atlantic²⁴. Because of the open connection between the Norway Basin and the Arctic Ocean, these events should also leave their imprints in the benthic record from the Arctic Ocean. We suggest that the benthic record published for core T3-67-11¹ might be easier to explain by shortening the time scale dramatically. The transition recorded at 180-150 cm depth in the core might have taken place around 13 kyr ago and the lower part of the core is perhaps not older than oxygen-isotope stage 4 (~ 72 kyr ago).

If we assume that the palaeomagnetic time frame is correct, then *Elphidium excavatum* must have been the dominant species on the shallow shelves bordering the Arctic Ocean in the late Pliocene or earlier. Little information exists concerning the benthic foraminifera on the shelf areas bordering the Arctic

Ocean during the Pliocene or early Pleistocene, but where *Elphidium excavatum* f. *clavata* occurs in faunas of this age elsewhere, it is not as dominant as in late Quaternary faunas. Such species as *Elphidiella hannah*, *Cibicides grossa*, *Cassidulina teretis*, *Elphidium oregonense* and Polymorphiniid-textuariid containing assemblages²⁵⁻²⁷ are common elements in shallow-water assemblages of the late Pliocene and early Quaternary, these are not recorded in short cores from the deeper parts of the Arctic Ocean. This might possibly be explained by environmental factors, but elsewhere late Quaternary sediments at high latitudes are dominated by *Elphidium excavatum* f. *clavata*²⁸⁻³².

We conclude that the biostratigraphy does not provide conclusive evidence about the age of the sediments in the Arctic Ocean cores, and that there are a certain features that indicate a late Quaternary rather than a Pliocene age for the lower portions of the cores.

The extent of isoleucine epimerization measured in planktonic foraminifera from Arctic Ocean core T3-67-11 cannot be reconciled with the proposed palaeomagnetic time scale. The alle/Ile ratio in a sample from 260-cm depth in the core suggests an age younger than 300 kyr, whereas the palaeomagnetic scale proposed an age of 3 Myr. We conclude that the published palaeomagnetic data are insufficient to exclude alternative age interpretations and that the micropalaeontology is equally, if not more compatible with a late Quaternary age for the entire core. The possibility that Arctic Ocean sedimentation rates are an order of magnitude or more greater than previously envisioned adds new interest to cores from this region. A rigorous study of palaeomagnetism, amino acid ratios and stable-isotope variations in conjunction with biostratigraphical and sedimentological investigations is required to resolve these discrepancies.

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Sequence of a cDNA clone encoding human preproinsulin-like growth factor II

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The insulin-like growth factors (IGF) I and II are single-chain serum proteins of 70 and 67 amino acids, respectively, which are synthesized by the liver and possibly other tissues¹⁻⁴. They are probably required for normal fetal and postnatal growth and development⁵⁻¹¹. They also stimulate the growth of cultured cells, possibly by controlling the progression through the G₁ phase of the cell cycle¹². In contrast to IGF-II whose concentration does not vary during postnatal development, the serum levels of IGF-I increase several-fold to adult levels during puberty¹³. The serum concentration of IGF-I is a sensitive monitor of growth hormone levels and is decreased in individuals with growth hormone deficiency and elevated in those with growth hormone-secreting tumours^{6,14}. As a first step in studying the biosynthesis of these proteins and elucidating their role(s) in normal development and in tumorigenesis, we have isolated and sequenced cDNAs prepared from adult human liver mRNA which encode the precursors to IGF-I and -II. We report here the sequence of a cDNA encoding a 180-amino acid protein which is the precursor to IGF-II.

IGF-specific clones were isolated from an adult human liver cDNA library¹⁵ by hybridization with a ³²P-labelled 256-fold degenerate 23-base oligonucleotide probe



whose sequence was derived from a region that is identical in human IGF-I (amino acids 46-53) and IGF-II (amino acids 45-52)^{1,2}. For eight of ~9,000 colonies hybridized with this probe, analysis of the sizes of the *Pst*I fragments indicated that four were different. The nucleotide sequences of the inserts of these four were determined and the predicted amino acid sequence in each reading frame compared with that of IGF-I and IGF-II. The inserts in plasmid phigf 1 and phigf 2 encoded IGF-I and -II, respectively. The proteins encoded by the other plasmids were not IGF-like; one encoded α_2 -macroglobulin. The identity of the fourth clone is unknown.

The plasmid phigf 1 contained an insert of ~660 base pairs (bp) which extended from the second nucleotide of the codon for amino acid -15 of the signal peptide of the IGF-I precursor to the poly (A) tract. The sequence was identical to that reported by Jansen *et al.*¹⁶ for another cDNA, which included the entire signal peptide and 80 bases of 5'-untranslated region, except that it lacked the two guanosine residues present in the poly(A) tract of their sequence.

The sequence of human preproIGF-II mRNA was deduced from the sequence of the insert in plasmid phigf 2 (Fig. 1). Translation of the mRNA from the first Met codon predicts the sequence of a 180-amino acid protein in which the 67-amino acid sequence of IGF-II begins 25 residues from the start (Fig. 1). Including the opal termination codon, the coding region is 543 bases long. The 5'-untranslated region comprises at least 250 bases and the 3'-untranslated region over 253 bases. The phigf 2 cDNA has neither a polyadenylation signal nor a poly(A)

CAGGGGCGAAGAGACACCGAGCUUGUGGGAGGAGGUGGAUCCAGCCCCAGGCCAGGCGUCUGAAUCGCGCAGCUCAGCCCCUGCCAGCCGCCACAGCCUGAGC	119
CCCAGCAGGCCAGAGAGCCAGUCUGAGGAGGAGCUGUGGGCUGUGGCCAGGCCAGCCAGCUCGAGGCGAGCCAGCCGCCACUCCGAGGC	238
AGAGAGACACCA	
Met Gly Ile Pro Met Gly Lys Ser Met Leu Val Leu Leu Thr Phe Leu Ala Phe Ala Ser Cys Cys Ile Ala Ala Tyr	
AUG GGA AUC CCA AUG GGG AAG UCG AUG CUG GUG CUU CUC ACC UUC UUG GCC UUC GCC UCG UGC UGC AUU GCU GCU UAC	328
Arg Pro Ser Glu Thr Leu Cys Gly Gly Gly Lys Val Asp Thr Leu Gln Phe Val Cys Gly Asp Arg Gly Phe Tyr Phe Ser Arg Pro Ala	
CGC CCC AGU GAG ACC CUG UGC GGC GGG GAG CUG GUG GAC ACC CUC CAG UUC GUC UGU GGG GAC CGC GGC UUC UAC UUC AGC AGG CCC GCA	418
Ser Arg Val Ser Arg Arg Ser Arg Gly Ile Val Glu Glu Cys Cys Phe Arg Ser Cys Asp Leu Ala Leu Leu Glu Thr Tyr Cys Ala Thr	
AGC CGU GUG AGC CGU CGC AGC CGU GGC AUC GUU GAG GAG UGC UGU UUC CGC AGC AGC UGU GAC CUG GCC CUC CUG GAG ACG UAC UGU GCU ACC	508
Pro Ala Lys Ser Glu Arg Asp Val Ser Thr Pro Pro Thr Val Leu Pro Asp Asn Phe Pro Arg Tyr Pro Val Gly Lys Phe Phe Gln Tyr	
CCC GCC AAG UCC GAG AGG GAC GUG UCG ACC CCU CCG ACC GUG CUU CCG GAC AAC UUC CCC AGA UAC CCC GUG GGC AAG UUC UUC CAA UAU	598
Asp Thr Trp Lys Gln Ser Thr Gln Arg Leu Arg Arg Gly Leu Pro Ala Leu Leu Arg Ala Arg Arg Gly His Val Leu Ala Lys Glu Leu	
GAC ACC UGG AAG CAG UCC ACC CAG CGC CUG CGC AGG GGC CUG CCU GCC CUC CUG CGU GCC CGC CGG GGU CAC GUG CUC GCC AAG GAG CUC	688
Glu Ala Phe Arg Glu Ala Lys Arg His Arg Pro Leu Ile Ala Leu Pro Thr Gln Asp Pro Ala His Gly Gly Ala Pro Pro Glu Met Ala	
GAG GCG UUC AGG GAG GCC AAA CGU CAC CGU CCC CUG AUU GCU CUA CCC ACC CAA GAC CCC GCC CAC GGC GGC GCC CCC CCA GAG AUG GCC	778
Ser Asn Arg Lys OP	
AGC AAU CGG AAG UGA GCAAAACUGCCGCAAGUCUGCAGCCCGGCCACCAGCUCGAGCCUCCUGACCACGGAGUUCUCCAGGUUCCAUCCCGAAAUUCUCUGGUUCCA	895
CGUCCCCUGGGGCUUCCUGACCCAGUCCCGGCCCGCCGAAACAGGCUACUCCUCGCGCCCGUCCUACGGGUGAGGAAGCACAGCAGCUUUAACAACUAGUACAAA	1,015
AUCGAUUGCCUUAAACACCUACAUACCU	1,046

Fig. 1 Sequence of human preproIGF-II mRNA and protein. The predicted amino acid sequence of preproIGF-II is numbered by designating the first amino acid of IGF-II as 1. The region corresponding to IGF-II is boxed and pairs of basic amino acids are underlined. The B-domain of IGF-II comprises residues 1–32, the C-domain comprises residues 33–40, the A-domain residues 41–61, the D-domain residues 62–67, and the carboxy-terminal E-domain residues 68–156. The number of the nucleotide at the end of each line is indicated.

Methods: 9,000 transformants from the adult human liver cDNA library of Woods *et al.*¹⁵ were grown in 96-well microtitre dishes. Colonies were transferred to Whatman 541 paper, grown, amplified with chloramphenicol and lysed as described elsewhere³². Colonies containing IGF sequences were identified by hybridization with a 256-fold degenerate 23-base oligonucleotide which had been labelled with [γ -³²P]ATP and T4 polynucleotide kinase³³. Oligonucleotides were made on an Applied Biosystems DNA Synthesizer (Model 380A) and purified by electrophoresis in an 8 M urea, 20% polyacrylamide gel. The filters were hybridized in 5×SSC (SSC = 0.15 M NaCl, 0.015 M sodium citrate), 50 mM sodium phosphate pH 7.0, 0.2% SDS, 2×Denhardt's³⁴, 200 μ g ml⁻¹ sonicated and denatured salmon sperm DNA and 10⁶ c.p.m. ml⁻¹ of ³²P-labelled oligonucleotide pool at 30 °C. After 16 h they were washed in 5×SSC, 0.1% SDS at 42 °C for 1 h. Hybridizing colonies were identified by autoradiography. The inserts in the plasmids phigf 1, 2, 4 and 5 were sequenced. The sequence of the inset in phigf 2 presented here was determined on both strands and across all restriction sites used to initiate sequence determinations, by the procedures of Maxam and Gilbert³³ and Sanger *et al.*³⁵.

tract. No other clones encoding preproIGF-II mRNA were revealed when the insert in phigf 2 was used as a probe to re-screen the original 9,000 colonies and 6,000 more. Attempts to determine the size of human preproIGF-II mRNA by hybridization to a Northern blot¹⁷ of human adult liver poly(A)⁺ RNA have been inconclusive, presumably because of its low abundance (< 1/10,000 mRNA molecules).

As IGF-II is a secreted protein, we believe that the 24-residue aminoterminal extension is the signal peptide. Analysis of the hydrophilicity of preproIGF-II as described by Hopp and Woods¹⁸ indicates that the putative signal peptide has a hydrophobic core of 14 residues (amino acids –15 to –2) and a profile similar to that of other signal peptides. Interestingly, about 25% of the purified human IGF-II molecules lack Ala 1 (ref. 2), suggesting that cleavage of the Ala –1–Ala 1 peptide bond by the peptidase is preferred but that the Ala 1–Tyr 2 bond is also cleaved. As ~50% of the rat IGF-II molecules also lack Ala 1 (ref. 19), this feature is not restricted to the human protein. A similar hydrophobic analysis of the preproIGF-I sequence reported by Jansen *et al.*¹⁶ suggests that the 25-amino acid segment adjacent to the amino-terminus of IGF-I is also a signal peptide and that translation is not initiated at the in-frame Met codon 69 bases upstream. Thus, human preproIGF-I probably comprises 130 amino acids.

Studies of the biosynthesis of rat IGF-II in a rat liver cell line have revealed several molecules which may be precursors to rat IGF-II^{19–22}, including rat preproIGF-II whose size, molecular weight (M_r) = 21,600, is similar to that reported here for the human precursor, M_r = 20,143.

Human proIGF-II has an 89-amino acid carboxyterminal extension, the E-domain, and proteolytic processing at Arg 68 is required to produce mature IGF-II. Proteolytic processing at single basic residues has been reported to occur in the generation

of other proteins including IGF-I¹⁶, epidermal growth factor²³ and growth hormone-releasing factor^{24,25}. However, processing occurs more often at pairs of basic amino acids²⁶, of which there are five in proIGF-II (Figs 1, 2) including one site within IGF-II that is not cleaved. It is unknown whether proteolysis occurs at the pairs of basic residues or at single basic amino acids within the E-domain, thus generating carboxy-terminal heterogeneity. Processing at such sites could explain the various size classes of IGF-II-like molecules secreted by cultured rat liver cells^{19–22}.

The function of the carboxy-terminal E-domain of proIGF-I or -II is unknown. Although the organization and sequences of the IGF moieties are similar, possessing 62% sequence identity², the carboxyterminal segments are of different lengths (Fig. 2) and show no amino acid sequence similarity. Steiner²⁷ has proposed that secretory protein precursors must maintain a minimum overall peptide chain length in order to be efficiently transferred into the endoplasmic reticulum during biosynthesis and it is possible that the carboxy-terminal segment of the IGF precursor is required for this process. The carboxy-terminal peptide could also confer unique physiological properties on the precursor or function as a separate entity. However, if the E-domain or proIGF-I and -II has a physiological function, the absence of sequence similarity suggests that it will probably be unique to each protein.

The IGFs insulin and relaxin are members of the insulin superfamily^{28,29}; their different structures are shown schematically in Fig. 2. The β -subunit of nerve growth factor and even the serine proteases may be more distantly related members of this family^{29,30}. These proteins possess significant similarity within the regions corresponding to the A and B chains of insulin^{1,2,28,29,31}. The sequences and sizes of the C-peptide region or domain are very different. In addition, the C-peptide of proinsulin and prorelaxin is excised during processing of the

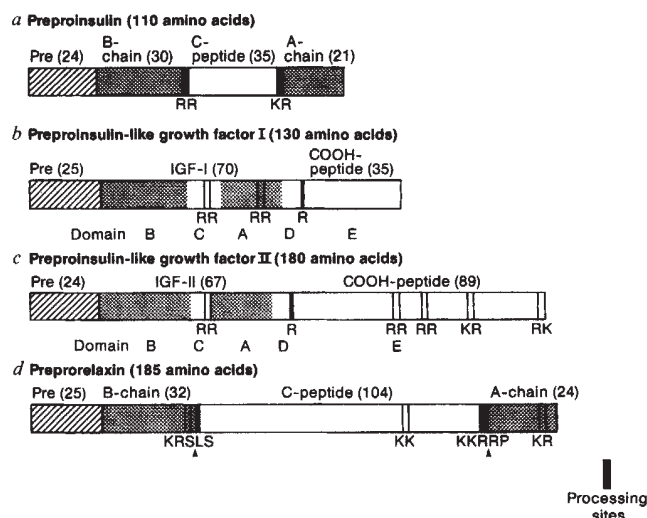


Fig. 2 Schematic representations of the structures of the precursors to human insulin, IGF-I, IGF-II and relaxin. The regions of each precursor are indicated and their sizes are given in parentheses. The stippled segments correspond to the homologous B- and A-chain regions or domains. Proteolytic processing sites and pairs of basic amino acids are indicated by solid and open boxes, respectively. The sequences of these sites are indicated by the one-letter code (K, lysine; L, leucine; P, proline; R, arginine; S, serine). The peptide bonds cleaved on processing of prorelaxin are indicated by arrowheads. The structure of preproinsulin is from Bell *et al.*³⁶; preproIGF-I, from ref. 16 and this report; preproIGF-II, this report; and preprorelaxin, ref. 31.

precursor but the C-domain is not removed from IGF-I or -II. The IGFs have acquired two additional domains, D and E, during their evolution.

The characterization of cDNAs encoding preproIGF-I and -II will facilitate an analysis of the role(s) of these proteins in normal growth and development and in oncogenesis. The cDNAs can be used as probes to identify tissues and cells which synthesize preproIGF-I and -II mRNA and to measure changes in mRNA levels during development. The synthesis of proIGF-I and -II in heterologous cells will provide material to determine whether these molecules have physiological roles other than their being precursors of IGF-I or -II.

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Insulin-like growth factor II precursor gene organization in relation to insulin gene family

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The insulin gene family^{1,2}, comprised of insulin, relaxin, insulin-like growth factors I and II (IGF-I and IGF-II) and possibly the β -subunit of 7S nerve growth factor³, represents a group of structurally related polypeptides whose biological functions have diverged. The IGFs, or somatomedins, constitute a class of polypeptides that have a key role in pre-adolescent mammalian growth (see ref. 4 for review). IGF-I expression is regulated by growth hormone^{5,6} and mediates postnatal growth, while IGF-II appears to be induced by placental lactogen during prenatal development⁶. The primary structures of both human IGFs have been determined and are closely related^{7,8}. A polypeptide highly homologous to human IGF-II is secreted by the rat liver cell line, BRL-3A^{9,10}. As this polypeptide, termed multiplication stimulating activity (MSA), differs from human IGF-II by only five amino acid residues, MSA probably represents the rat IGF-II protein¹¹. Using molecular cloning techniques, we have isolated cDNA and chromosomal genes coding for the MSA and human IGF-II precursors, respectively. Our data, presented here, indicate that both MSA¹² and human IGF-II are synthesized initially as larger precursor molecules. The deduced prohormones both have molecular weights (MWs) of 20,100 and contain C-terminal propeptides of 89 amino acid residues, which we have named E-peptides. The organization of the IGF-II precursor gene is discussed in relation to that of other insulin gene family members.

To isolate cDNA clones coding for the IGF-II precursor, we used an approach similar to that of Gray *et al.*¹³ for the cloning of mouse EGF precursor cDNA. As a first step, buffalo rat liver cells (BRL-3A) were used as the mRNA source for the cloning of MSA cDNA. We assumed that the nucleotide sequence of MSA and IGF-II mRNA would be sufficiently conserved to allow the use of an MSA cDNA hybridization probe for the subsequent isolation of IGF-II DNA sequences. As described in Fig. 1, we first cloned and characterized a specifically primed MSA cDNA fragment (pMSA107), which was subsequently used as a probe for the isolation of additional MSA cDNA clones from oligo(dT) and specifically primed BRL cDNA libraries¹³. The 1,735-base pair (bp) nucleotide sequence derived from clones pMSA-107, pMSA-19G3, pMSA-52H9 and pMSA-dT9 is shown in Fig. 2a.

Translation of the sequence into amino acid sequence information reveals an open reading frame of 392 residues which

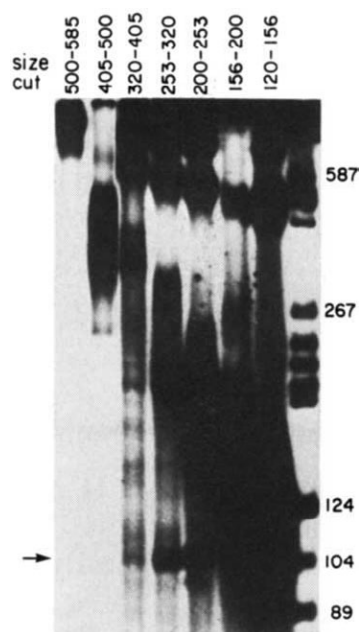


Fig. 1 Cloning of the MSA-specific *AluI* fragment. The arrow indicates the 107-bp specifically primed *AluI* fragment.

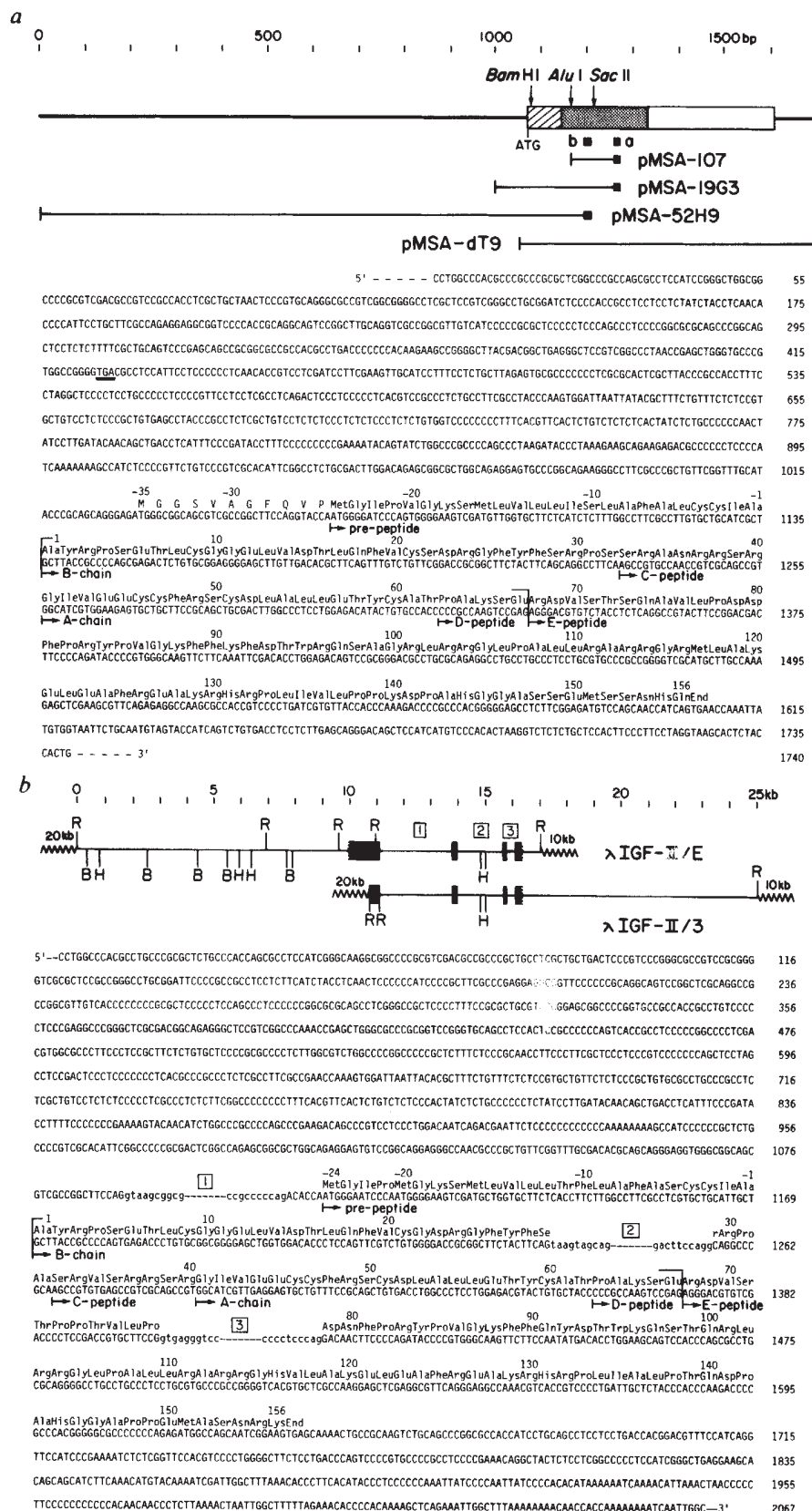
Methods: The synthetic primer pool²⁶ (3'-AA⁺CA⁺CA⁺TC⁺TC⁺5') (480 pmol) complementary to sequences between amino acids 44 and 48 of buffalo rat liver cell MSA was radiolabelled using T4 polynucleotide kinase and [γ -³²P]-ATP (>5,000 Ci mmol⁻¹) to a specific activity of $\sim 0.5 \times 10^6$ c.p.m. pmol⁻¹ and subsequently used as a primer for the synthesis of otherwise unlabelled single-stranded cDNA. The ³²P-labelled primers were ethanol-precipitated with 20 μ g poly(A)⁺ RNA which had been isolated from a line of normal buffalo rat liver cells, BRL-3A, clone BRL-T (a gift from G. Todaro), by the guanidine thiocyanate-CsCl procedure²⁵, followed by oligo(dT)-cellulose chromatography. The pellet was dissolved in 50 μ l of 100 mM KCl and the mixture annealed by incubating for 5 min at 90, 68, 42 and 37 °C, followed by the addition of all four deoxy-oligonucleotides (500 μ M) and reverse transcriptase for first strand cDNA synthesis. The second strand was synthesized under standard conditions¹³. After treatment with pancreatic ribonuclease A, phenol-chloroform extraction and ethanol precipitation, the cDNA was fractionated on a 6% polyacrylamide gel. The gel was cut into 0.5-cm slices and the cDNA eluted by crushing and soaking the gel slice in 500 mM ammonium acetate, 10 mM Mg acetate, 1 mM EDTA, 0.1% SDS at 37 °C overnight. The eluate was ethanol-precipitated and one-sixth of each fraction digested in parallel with restriction endonucleases *HaeIII* or *AluI*. Computer-assisted analysis of the MSA amino acid sequence allowed us to calculate the sizes of all possible MSA cDNA fragments which could be generated by our labelled primer after cleavage with the two restriction enzymes (30, 40 and 107 bp for *AluI* and 40, 45, 53, 134 and 142 bp for *HaeIII*). The digestion products were analysed on an 8% polyacrylamide gel, 1.2 mm thick and 40 cm long. ³²P-labelled pBR322 *HaeIII* fragments were used as size markers. The *AluI* digest generated a fragment of the expected size (107 bp) and the same cDNA fractions (200–405 bp) yielded a 53-bp *HaeIII* fragment (not shown) also of the expected size. This result strongly suggested that these fragments were derived from MSA cDNA. We concentrated on the cloning of the 107-bp *AluI* fragment because of its larger size. The remainder of the cDNA fractions in the size range 200–405 bp were pooled, cut with *AluI* and fractionated as above. The gel was exposed to X-ray film for 3 days without drying at -70 °C with an intensifying screen (Cronex Lightning Plus). The 107-bp fragment was electrophoretically extracted (0.5 ng) and cloned into the *PstI* site of pBR322 by a standard G/C tailing procedure. 212 clones were obtained and subsequently screened using the ³²P-labelled primer pool (see above) as hybridization probe. Of the two resulting positives, one was completely sequenced by the Maxam-Gilbert method¹⁹. Clone pMSA-107 contained the expected MSA coding sequence, beginning at the 3'-end with the complementary primer sequence, 5'-GAAGAGTGTCTT-3', and ending at the 5' end with nucleotides C and T, which comprise the 3' half of the *AluI* recognition sequence.

contains the 67-amino acid MSA sequence; 201 codons of this open reading frame are probably part of the 5'-untranslated region of MSA mRNA. The high G + C content of this part of the sequence is the probable reason for the lack of in-frame termination codons, which usually occur just upstream from an initiator methionine codon. Three potential initiation codons are found at positions -16, -24 and -35 upstream from the amino-terminal residue of mature MSA (Ala, position 1; Fig. 2a). Downstream from the carboxy-terminal glutamic acid residue¹¹ (position 67), the open reading frame extends for another 89 codons. This C-terminal extension is presumably a propeptide, which we have designated E-peptide. The calculated molecular weights of the three possible preproMSA sequences are 21,100 (Met -35), 20,100 (Met -24) and 19,300 (Met -16). *In vitro* translation experiments reported previously had suggested a 21,600-MW MSA precursor¹², which would make Met -35 a strong candidate for the initiator position. Met -24, however, is followed by a stretch of amino acids bearing features common to signal peptides, necessary for the translocation of secreted polypeptides into the lumen of the endoplasmic reticulum. Signal sequences range in size from 15 to 30 amino acids and usually contain a central hydrophobic stretch of amino acids flanked by polar and charged residues. Examination of the amino acid sequence preceding the amino-terminus of MSA with regard to these structural criteria suggests Met -24 as the initiator methionine. The fact that this is precisely the length of the homologous preproinsulin signal peptide^{14,15} supports this tentative assignment. The calculated MW of preproMSA beginning at Met -24 is also in reasonably good agreement with the value determined by *in vitro* translation analysis. Should Met -35 be the true amino-terminus of preproMSA, this protein would contain the longest signal peptide described to date.

The 540 nucleotide-long sequence coding for the putative 180-amino acid preproMSA polypeptide is flanked by 137 nucleotides of 3'- and 1,059 of presumably 5'-untranslated sequences. Neither untranslated region appears to be complete. Due to difficulties which we believe were caused by the extremely high G + C content in the 5' region (>70%) and possibly a similar structural barrier in the 3' sequence of MSA mRNA, we did not succeed in cloning the entire mRNA complement.

To obtain detailed information regarding the organization of the human IGF-II chromosomal gene, MSA cDNA probes were used to screen a fetal liver λ DNA library^{16,17}. We obtained two recombinant λ clones that contained overlapping fragments of human DNA, together representing about 25 kilobases (kb) of chromosomal DNA (λ IGF-II/E and λ IGF-II/3; Fig. 2b). Characterization of these clones by physical mapping, Southern blot hybridization¹⁸ and DNA sequence analysis¹⁹ allowed us to establish the intron/exon topography and nucleotide sequence of the entire coding portion of the IGF-II gene, and large parts of its transcribed, but presumably untranslated, sequences (Fig. 2b). IGF-II gene sequences matching our MSA cDNA sequence are interrupted by three introns. We have recently isolated and characterized a human IGF-II cDNA clone (data not shown) from a fetal liver cDNA library which enabled the designation of 3' sequences further downstream as untranslated sequences. Joining of the four exon sequences at splice donor and acceptor sites yields a 2,066-bp nucleotide sequence. The open reading frame containing the known 67-residue amino acid sequence of IGF-II⁷ is 272 codons long. As in the MSA sequence, few Met codons are found in this open reading frame because of its low A + T content.

Upstream from residue -1 (alanine) of IGF-II, ATG codons are found at positions -16, -20 and -24; two of these (-16 and -24) are conserved between MSA and IGF-II sequences. Comparison of MSA and IGF-II putative pre-sequences further supports the assignment of Met -24 as the initiator methionine, for the same reasons described earlier (Fig. 3). Only four amino acids in their putative signal sequences (16%) are not conserved between rat and human IGF-II. Despite these differences the overall characteristics common to signal peptides are unchanged²⁰. These amino acid changes were all caused by



position 33 of MSA instead of the reported glycine. This reduces the number of differences between MSA and IGF-II to only four (~6%). At the carboxy-terminus of IGF-II, the open reading frame extends for another 267 nucleotides, coding for a propeptide (E-peptide; see Figs 2b, 3) of the same length as the propeptide in proMSA (89 amino acids). The two E-peptides display only 19 differences (21%; Fig. 3). The IGF-I precursor

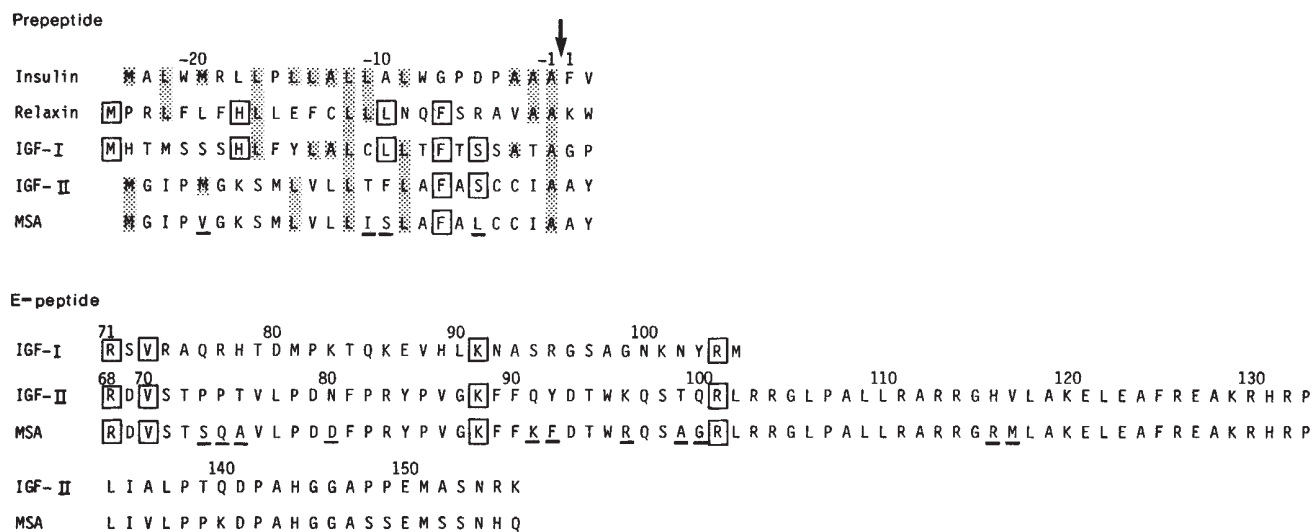


Fig. 3 Comparison of the prepeptide and E-peptide domains of insulin gene family precursor proteins. The previously unknown amino acid sequences of MSA and IGF-II precursor domains are aligned. Shaded residues match those of the insulin prepeptide. Boxes indicate identities between other members of the insulin gene family. The arrow indicates signal peptidase cleavage sites. Underlining indicates residue differences between MSA and IGF-II.

also has a C-terminal propeptide²¹, which is 35 amino acids long and shows minimal homology with the E-peptide domains of IGF-II and MSA. This homology is restricted to sequences flanking the cleavage site from the mature growth factor (R-S-V as opposed to R-D-V in MSA and IGF-II; see Fig. 3). The carboxyl-terminal sequence extensions of MSA IGF-II and IGF-I may possess biological activity as has been shown for pro-calcitonin²². This possibility is supported by the high degree of sequence conservation (79%) in this domain between MSA and IGF-II. Remarkably, even the long 5'-untranslated nucleotide sequences of MSA cDNA and the IGF-II gene are highly conserved (~80%) when sequences are aligned to compensate for insertions or deletions. This may also indicate some functional importance of this region of rat and human IGF-II mRNAs.

When comparing the IGF-II prepeptide sequence with those of other insulin gene family members (Fig. 3) one finds that residue -12 (Leu) and alanine (-1) at the signal peptidase cleavage site are conserved in all members. IGF-II (Met -24) and insulin^{14,15} prepeptides are of identical length (24 amino acids) and those of IGF-I²¹ and relaxin²³ are each 25 residues long. Thirteen residues (54%) of the proinsulin prepeptide are conserved in at least one of the other members, residues -4, -5 and -6 being the most variable ones (Fig. 3).

Interestingly, three of the four chromosomal genes of this gene family that have been at least partially characterized (insulin¹⁵, relaxin²³, IGF-I²⁴ and IGF-II) contain intervening sequences which interrupt 5'-untranslated sequences shortly before the initiation codon, further substantiating a common evolutionary origin (see Fig. 4). The intron interrupting the C-terminal propeptide of IGF-II (at position 78), however, is

not present in the preproinsulin and relaxin genes and has not yet been demonstrated for the IGF-I gene. The third intervening sequence, which is located at similar positions in the insulin and relaxin genes²³ close to the amino-terminus of the C-peptide, is found in IGF-I and IGF-II genes interrupting a sequence (at position 26 of IGF-I and 29 of IGF-II) which has been designated as the carboxy-terminus of the B-chain. The rather low level of sequence homology with the B-chain of insulin in this area seems to favour a redefinition of the C-terminus of IGF-I and IGF-II B-chains to positions 23 and 28 of IGF-I and IGF-II, respectively, which would place the intron at the junction between the B and C domains, and further support the ancestral relationship with insulin¹⁵ and relaxin²³ genes. The difference in length between IGF-I and IGF-II C-peptide domains is caused by a 12-bp insertion or deletion between Ala -32 and Ser -33 of IGF-II.

The BRL cell line that was used as a source of mRNA has been reported to secrete polypeptides of 16,300, 8,700 and 7,100 MW which are immunologically related to MSA¹². These related polypeptides may represent processing intermediates of preproMSA, translation products of variant mRNAs transcribed from the same or rearranged gene or products of related but as yet unknown genes. Aside from the obvious endopeptidase cleavage sites at the amino- and carboxy-termini of rat and human IGF-II, Arg-Arg dipeptides are present in the E-peptides—these are known to be potential cleavage sites for polypeptide precursors. Determination of terminal amino acid sequences will be necessary to identify these IGF-II-related polypeptides.

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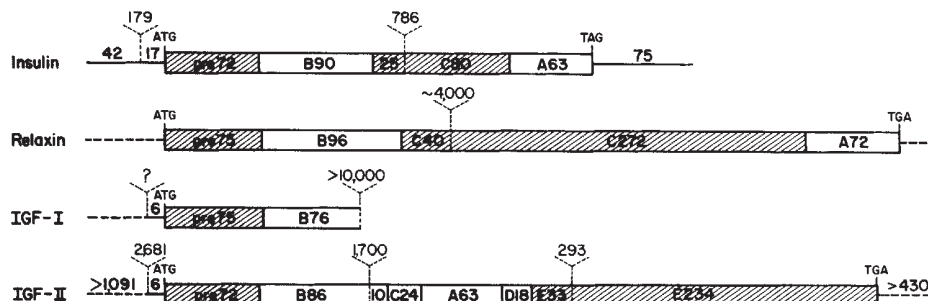


Fig. 4 Intron-exon organization of insulin-related chromosomal genes. Open boxes designate domains that are present in mature polypeptides and cross-hatched boxes those that are absent. Numbers within boxes and on forks indicate the length in nucleotides of coding sequences and introns, respectively. Numbers on lines indicate the lengths of untranslated regions.

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Human chromosomal mapping of genes for insulin-like growth factors I and II and epidermal growth factor

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Many of the actions previously attributed to pituitary-derived growth hormone are mediated by polypeptide growth factors^{1–3}. These include the insulin-like growth factors I and II (IGF-I and IGF-II)^{4–6}, which are members of the insulin family of proteins⁷. We report here the chromosomal mapping of the human genes for IGF-I and IGF-II. IGF-II maps to the short arm of chromosome 11, which also contains the gene for insulin⁸ and the proto-oncogene *c-Ha-ras1* (ref. 9). IGF-I maps to chromosome 12, which is evolutionarily related to chromosome 11 and carries the gene for the proto-oncogene *c-Ki-ras2* (refs 10, 44). We have also localized the human gene for an unrelated polypeptide hormone, epidermal growth factor, to chromosome 4q, in the same region as another specialized growth factor, T-cell growth factor¹¹. We speculate that these map assignments reflect the existence of gene families involved in growth control.

IGF-I and IGF-II are structurally very similar. IGF-I contains 70 amino acids and is identical to somatomedin C. IGF-II is about the same size as IGF-I (67 amino acid residues) and may be identical to somatomedin A⁴. IGF-I has recently been demonstrated to stimulate a tyrosine-specific protein kinase in a manner similar to that of many other mitogens^{12,13} and some oncogenes^{14,15}. Epidermal growth factor (EGF), which is unrelated to the insulin-like growth factors, is produced by the salivary gland as a single chain polypeptide hormone 53 amino acids long¹⁶. The physiological function of EGF is not completely understood, but the peptide is known to stimulate epidermal growth and keratinization in many tissues.^{16,17} Human EGF may be identical to β -urogastrone, a gastric antiseecretory hormone found in human urine¹⁶.

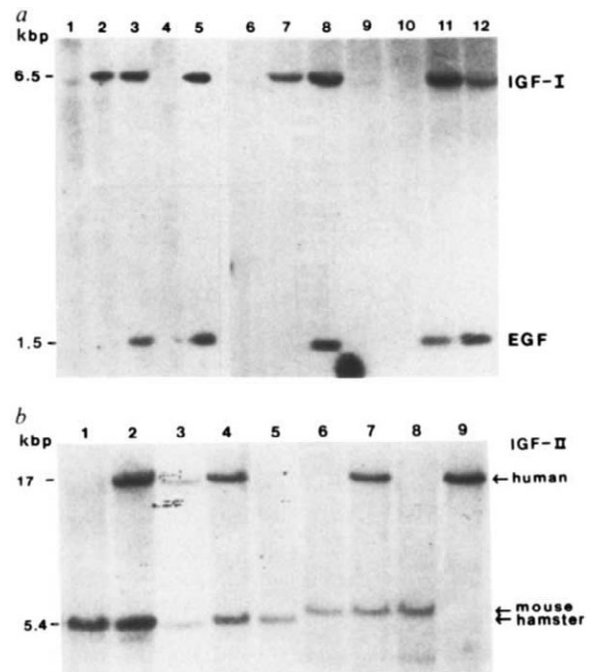


Fig. 1 Hybridization of *a*, human EGF and IGF-I and *b*, human IGF-II probes to human, rodent and hybrid DNA. *a*, Human control DNA (lane 5) produced a single *EcoRI* band each with both IGF-I and EGF probes while Chinese hamster DNA (lane 4) generated a weak band with IGF-I (slightly smaller than the human band) and no cross-hybridization with EGF. Chinese hamster/human hybrids in lanes 3, 8, 11 and 12 hybridized with both probes, while hybrids in lanes 2 and 7 were positive for IGF-I but negative for EGF. Hybrids in lanes 1, 6, 9 and 10 were negative for both human fragments, but the Chinese hamster IGF-I fragment was visible on the autoradiograms. All lanes received 10 μ g DNA except lane 5 which received 5 μ g. *b*, Regional mapping of IGF-II on human chromosome 11. Hybrids in lanes 1–4 were derived from Chinese hamster cells (lane 5) and a human donor with a t(11;15) (p11;p12) translocation²² (lane 9). The hybrid in lane 4 had retained both translocation chromosomes in the absence of the normal 11 and the hybrids in lanes 2 and 3 had retained only the derivative 15 chromosome with the short arm of chromosome 11. All three hybrids were positive for IGF-II. The hybrid in lane 1 had retained the reciprocal product of the translocation with the long arm of chromosome 11 and was negative. The mouse/human hybrid in lane 7 contained region p11→p15 of human chromosome 11 rearranged with chromosome 17 and maintained under hypoxanthine–aminopterin–thymidine selection pressure⁴⁷. The hybrid in lane 8 is a derivative that has lost this human chromosome and does not have the human IGF-II restriction fragment. Both mouse 3T3 (lane 6) and hamster (lane 5) cross-hybridized with the human IGF-II probe. All lanes were loaded with 6 μ g DNA, except for lane 3 which received only 3 μ g.

Methods: Samples in *a* were cleaved to completion with *EcoRI* (New England Biolabs) and those in *b* with *BamHI* (International Biotechnologies, Inc.). The genomic fragments were ³²P-labelled by nick-translocation³⁸. Probes were hybridized^{39,40} under stringent conditions (67 °C and 5 × SSC for EGF and IGF-I; 68 °C and 4 × SSC for IGF-II).

We have used human genomic DNA fragments that are homologous to the IGF-I, IGF-II and EGF genes^{18,19} in Southern blotting experiments. Genomic DNA was extracted, as described previously²⁰, from 26 human/rodent somatic cell hybrid clones that contained subsets of human chromosomes defined by chromosome analysis, enzyme marker and DNA marker studies. The hybrids were derived from six different hybrid series with Chinese hamster or mouse 3T3 fibroblasts as the rodent parents. The human cells, either fibroblasts or leukocytes, were all heterozygous for defined chromosomal rearrangements^{21–23}.

The IGF-I probe was a 3.1-kilobase (kb) subclone containing the 5' exon of the IGF-I sequence from phage λ Ch4A/IGF-I/A¹⁸. When hybridized to *EcoRI*-cleaved genomic DNA, the probe produced a single band of ~6.5 kb (Fig. 1*a*, lane 5). A

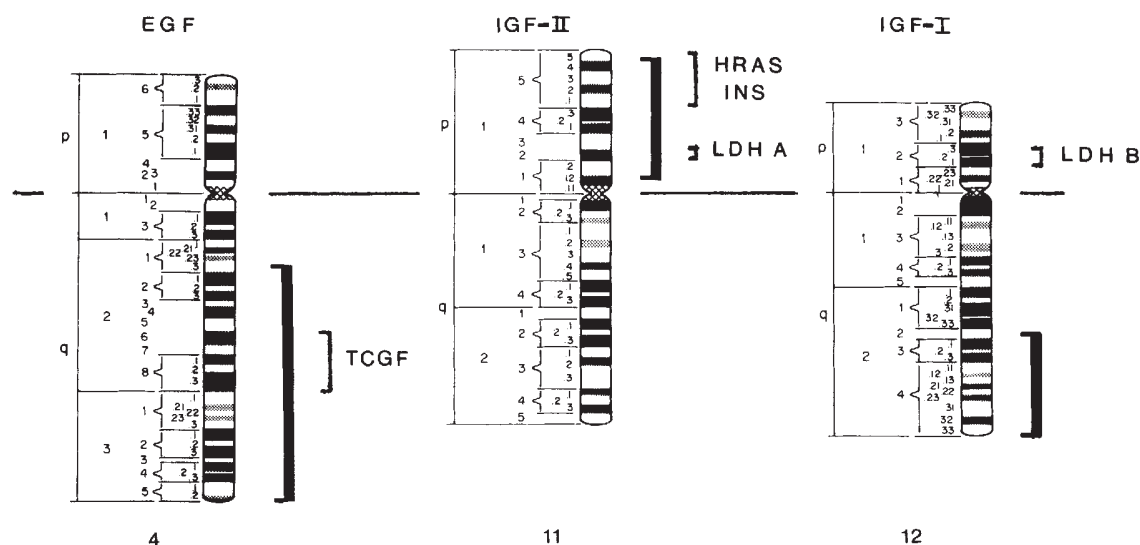


Fig. 2 Localizations of the human genes for growth factors reported here (heavy brackets). Light brackets indicate the locations of other growth-related genes as they coincide with the regional assignments reported here.

cross-hybridizing Chinese hamster band, slightly smaller in size and weaker in intensity than the human band, was easily distinguished (Fig. 1a, lane 1). The presence or absence of the human band correlated with human chromosome 12 in all 21 hybrid clones studied. For all other human chromosomes there were between 4 and 14 discordancies between the presence or absence of the human fragment and the human chromosome (Table 1). No human band was detected in a hybrid clone that contained a chromosome 12 with deletion of the distal long arm region (Fig. 1a, lane 6), which suggests that the gene for IGF-I is localized on that part of the chromosome (region 12q22 → qter; see Fig. 2).

The IGF-II probe, a 4.3-kb subclone containing the first two exons of the human IGF-II sequence¹⁹, produced a single dark band of ~17 kb when hybridized to *Bam*HI-digested human genomic DNA (Fig. 1b, lane 9). Rodent DNA produced major bands much smaller than the human band (~5.4 kb for Chinese hamster and ~6 kb for mouse; Fig. 1b, lanes 5 and 6). These cross-hybridizing bands served as internal controls in hybrids that were negative for the human sequence. In 17 hybrids studied, the human band was present only when human chromosome 11 was present and was not detected in the absence of chromosome 11. All other human chromosomes were excluded by four or more discordant hybrids (Table 1).

The gene for IGF-II was regionally localized on chromosome 11 using hybrid clones containing defined parts of chromosome 11 (Fig. 1b). The positive hybridization signal for human IGF-II correlated with the presence of the short arm of chromosome 11 (Fig. 1b, lanes 2, 3, 4 and 7). The signal was absent when only the long arm was present (Fig. 1b, lane 1). These results suggest that the gene for human IGF-II resides in region 11p15 → 11p11, the same region to which we have previously assigned the human loci for insulin and *c-Ha-ras1* (refs 9, 47) (see Fig. 2).

To map the human EGF gene, we used a 1.5-kb genomic *Eco*RI fragment that contains about 50% of the EGF coding region subcloned in pBR325 (A.U., in preparation). When hybridized to *Eco*RI-cleaved genomic DNA, the EGF probe generated a single band 1.5 kb in size, corresponding to the human EGF gene sequence (Fig. 1a, lane 5). No cross-hybridization with the Chinese hamster DNA was detected under these conditions (Fig. 1a, lane 4). After the fragment sizes for IGF-II and EGF had been established individually, both probes were hybridized simultaneously to the same filters (Fig. 1a). There was discordancy between the two growth factor genes, with some hybrids containing only one or the other band. The human EGF band was only present when human chromosome 4 had been retained (Fig. 1a, lanes 3, 8, 11 and 12) and was absent

when chromosome 4 was absent (Fig. 1, lanes 1, 2, 6, 7, 9 and 10). The fact that the EGF-negative hybrids in lanes 2 and 7 were positive for IGF-I on the same filters served as internal controls. There was no discordancy between chromosome 4 and the human EGF band in 22 informative hybrids (Table 1). All other human chromosomes were discordant in 4–14 hybrids. Two Chinese hamster/human clones containing only portions of chromosome 4 provided regional mapping information. Both hybrids, containing either region 4q21 → qter or 4cen → qter (Fig. 3), were positive for the human EGF gene sequence (Fig. 1a, lanes 3 and 11). These results allow us to assign the human gene for EGF to the distal portion of the long arm of chromosome 4 (bands 4q21 → qter) (see Fig. 2).

The only other growth factor genes previously mapped to human chromosomes are platelet-derived growth factor (PDGF; chromosome 22), localized as a result of its demonstrated homology to the proto-oncogene *c-sis* (ref. 24), the β -subunit of nerve growth factor to band 1p22 (ref. 25) and T-cell growth factor (also called interleukin-2) to chromosome 4q¹¹. Our assignments of the genes for EGF, IGF-I and IGF-II to human chromosomes (Fig. 2) therefore double the number of known human growth factor map locations.

We have assigned IGF-II to the same region (11p11 → p15) that contains the insulin locus. Divergence of insulin-like growth factors and proinsulin from a common ancestor has been suggested on the basis of their considerable sequence homology^{5,6}. It remains to be seen whether the genes are as close to each other as are the members of the β -globin gene cluster.

The mapping of the structurally related growth factor genes *IGF-I* and *IGF-II* to human chromosomes 11 and 12 adds to the existing evidence that these two chromosomes may be evolutionarily related, as suggested by the similarity in their banding patterns. Chromosome 11 contains genes for insulin, lactate dehydrogenase A and *c-Ha-ras1* on the short arm, and chromosome 12 carries the gene for lactate dehydrogenase B on the short arm and *c-Ki-ras2*, which has not been regionally mapped. There is conflicting evidence that assigns *c-Ki-ras2* to the short arm⁴⁵ or to the long arm⁴⁶. Our assignments of *IGF-I* to chromosome 12, and of *IGF-II* to chromosome 11, thus add two more structurally related genes to these complementary groups of loci. It has been suggested that the human chromosomes 11 and 12 were derived from a common ancestor by an early tetraploidization event²⁶. The positioning of *IGF-II* on the short arm of chromosome 11, and the tentative localization of *IGF-I* on the long arm of chromosome 12, suggests the possibility that a pericentric inversion or intrachromosomal shift has altered the gene organization on chromosome 12 compared

Table 1 Correlation of human *EGF*, *IGF-I* and *IGF-II* sequences with human chromosomes in Chinese hamster × human somatic cell hybrids

Gene	Hybridization/ chromosome	Human chromosomes*																						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
<i>IGF-I</i>																								
	+/+	4	7	13	6	5	8	3	9	3	4	10	12	7	10	7	9	5	8	5	7	11	8	7
	-/-	8	9	6	8	5	5	7	2	8	9	5	9	5	3	5	6	6	6	5	5	3	3	4
	+/-	9	6	0	6	9	4	8	5	10	10	3	0	7	2	5	5	9	4	9	5	3	5	3
	-/+	1	1	4	2	5	5	1	7	1	1	3	0	5	5	5	4	4	4	5	4	6	7	3
	Discordant	10	7	4	8	14	9	9	12	11	11	6	0	12	7	10	9	13	8	14	9	9	12	6
	Total hybrids	22	23	23	22	24	22	19	23	22	24	21	21	24	20	22	24	24	22	24	21	23	23	17
<i>IGF-II</i>																								
	+/+	1	6	10	4	6	6	3	9	2	4	12	8	6	8	6	6	6	7	5	9	9	7	6
	-/-	4	6	2	5	5	3	5	2	4	7	5	4	3	0	2	3	5	4	4	4	2	2	1
	+/-	11	7	2	7	7	5	7	4	11	9	0	3	7	4	5	7	7	4	8	3	4	5	4
	-/+	3	1	5	2	2	4	0	5	1	0	0	3	4	4	5	4	2	3	3	1	4	5	2
	Discordant	14	8	7	9	9	9	7	9	12	9	0	6	11	8	10	11	9	7	11	4	8	10	6
	Total hybrids	19	20	19	18	20	18	15	20	18	20	17	18	20	16	18	20	20	18	20	17	19	19	13
<i>EGF</i>																								
	+/+	4	5	8	8	5	6	4	7	4	4	6	8	4	8	7	9	5	7	6	6	8	9	6
	-/-	12	11	5	14	9	7	9	5	12	13	5	7	6	4	7	10	10	7	10	7	4	7	7
	+/-	5	4	1	0	5	2	6	2	6	6	3	2	6	1	3	1	5	3	4	3	2	1	0
	-/+	1	3	9	0	5	7	0	9	0	1	7	4	8	7	5	4	4	5	4	5	9	6	4
	Discordant	6	7	10	0	10	9	6	11	6	7	10	6	14	8	8	5	9	8	8	8	11	7	4
	Total hybrids	22	23	23	22	24	22	19	23	22	24	21	21	24	20	22	24	24	22	24	21	23	23	17

* Only intact human chromosomes present in at least 20% of cells were included. Data on rearranged chromosomes or chromosomes present in less than 20% of cells were excluded.

with 11; the short arm/long arm ratio difference and the banding patterns support this hypothesis (Fig. 2). It will be of interest to determine whether this possible evolutionary rearrangement also involves the c-Ki-ras2 locus.

Comparative mapping studies, summarized in ref. 27, have localized the mouse genes that are homologous to c-Ha-ras1, insulin, lactate dehydrogenase A and the β -globin cluster (all located on human 11p) to the centre region of mouse chromosome 7, indicating evolutionary conservation of this linkage group. In contrast, the region containing the active c-Ki-ras2, lactate dehydrogenase B, triose phosphate isomerase and glyceraldehyde phosphate dehydrogenase genes, localized on human chromosome 12, is on mouse chromosome 6 (ref. 27). We predict that the mouse homologues of *IGF-I* and *IGF-II*, as new members of these conserved syntenic groups, will be found on mouse chromosomes 6 and 7, respectively.

The physical proximity of the proto-oncogene c-Ha-ras1 and the growth factor gene *IGF-II* on human chromosome 11, and the presence of the active c-Ki-ras2 gene together with *IGF-I* on chromosome 12, are intriguing because we have previously mapped another potential member of the insulin gene family²⁸, the β -subunit of nerve growth factor, and a distant relative of the *ras* gene family, *N-ras*, to the same band on the short arm of chromosome 1 (1p22)^{25,29,48}. The gene for relaxin (also related to insulin) has not yet been assigned to a chromosome. The three instances of synteny, or physical closeness, of a member of the *ras* gene family and an insulin family member that we have observed may have functional and/or evolutionary significance. For example, activation of the c-Ha-ras1 gene might influence *IGF-II* expression, in a similar way to the cooperation between *erb-A* and *erb-B*³⁰, to produce a fully transformed phenotype. *IGF-I* interacts with PDGF in the stimulation of competent BALB/c 3T3 cells and human fibroblasts to initiate DNA synthesis¹.

The gene for EGF maps to the same region of chromosome 4q as the gene for T-cell growth factor, a specialized growth factor produced by stimulated T-lymphocytes and T-cell lymphoma lines¹¹. Possibly EGF and T-cell growth factor form the nucleus of a gene family coding for peptides with similar functions. The nucleotide sequence of the EGF receptor is similar to that of the viral oncogene *v-erb-B*³¹, which encodes a glycoprotein related to tyrosine-specific protein kinases¹⁵. The gene for the human EGF receptor^{32,33} and the human gene sequence

homologous to *v-erb-B*³⁴ have been independently assigned to chromosome 7. The EGF receptor is therefore not encoded on the same chromosome as its ligand, as has been demonstrated for the transferrin receptor and transferrin^{35,49}, and for the low density lipoprotein receptor and apolipoprotein E^{36,37}.

The mapping of three important human growth factor genes, *EGF*, *IGF-I* and *IGF-II*, to defined regions of human chromosomes 4, 12 and 11 has raised interesting speculations with regard to the evolution of gene families involved in growth control, and of human and mammalian chromosomes. Experiments can now be designed to look for functional and evolutionary relationships between proto-oncogenes and these growth factors.

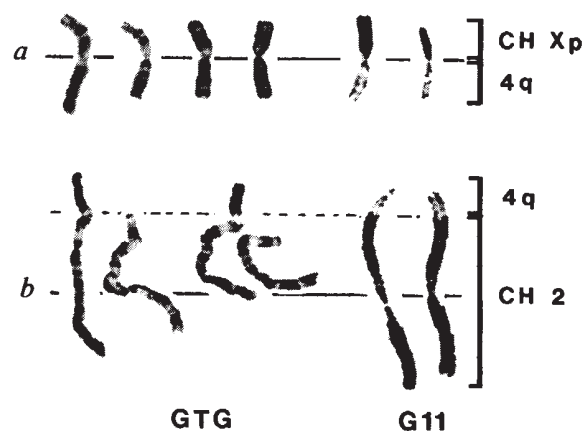


Fig. 3 Regional mapping of the gene for EGF on chromosome 4. Interspecies translocation chromosomes from Chinese hamster (CH)/human somatic cell hybrids, G-banded after trypsin treatment (GTG)⁴¹ or stained with alkaline Giemsa (G11)⁴² which distinguishes human (light) from rodent (dark) chromatin. *a*, Centromeric fusion of the short arm of CH X-chromosome with the long arm of human chromosome 4 in the hybrid shown in lane 11 of Fig. 1a. *b*, Translocation of the distal long arm of human chromosome 4 (region 4q21 → 4qter) onto the short arm of CH chromosome 2 in the hybrid shown in lane 3 of Fig. 1a⁴³. As both of these hybrids were positive for human EGF, the gene can be localized to the region 4q21 → qter.

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Localization of insulin-like growth factor genes to human chromosomes 11 and 12

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The insulin-like growth factors IGF-I and IGF-II are required for growth and development^{1–4}. Both are single-chain proteins (of 70 and 67 amino acids respectively) derived from precursors by proteolytic processing^{5–6}. IGF-I may be particularly important in promoting normal stature⁷ and IGF-II may be a fetal growth hormone⁸. The IGF proteins are probably synthesized by many normal tissues⁹ and by some tumours¹⁰. The secretion of growth factors by tumours and tumour-derived cell lines suggests that they may act as autocrine regulators of cell proliferation¹¹. Because of the possible role of these proteins in growth disorders

and cancer, and their sequence homology with insulin, we have determined their chromosomal localization. Using somatic cell hybrids¹² and cloned cDNA probes⁶ for these proteins, we have assigned the genes for IGF-I and IGF-II to human chromosomes 12 and 11, respectively. We present evidence that the IGF-II gene is located on the short arm of chromosome 11 with a *ras* proto-oncogene and the insulin structural gene, and also suggest the existence of a fragment length polymorphism using the IGF-I probe.

Cells from seven unrelated human and three mouse cell lines were fused and somatic cell hybrids selected and maintained as described previously¹³. On the same cell passage, cell hybrids were analysed for human chromosome-specific enzyme markers¹², karyotyped by Giemsa-trypsin staining¹⁴ and DNA was isolated for Southern filter hybridization¹⁵. The sequences coding for the IGFs were purified from an adult human liver cDNA library¹⁶. The probes used were the plasmids phigf1 and phigf2 (ref. 6) which encode preproIGF-I and preproIGF-II, respectively. Sequencing confirmed the presence of plasmids containing either a 660-base pair (bp) insert coding for IGF-I or a 1,046-bp insert coding for IGF-II (ref. 6). DNA (10 µg) was cut with the appropriate restriction endonuclease, then the DNA fragments were separated by electrophoresis on 0.8% agarose gels and transferred to nitrocellulose¹⁷. The cDNA probes were nick-translated using ³²P-labelled dNTPs and hybridization and autoradiography were done as described previously¹⁷.

The human insulin-like growth factor I gene (IGF-I) was mapped by correlating the presence of the gene with a specific human chromosome in a human-mouse hybrid cell panel. Human chromosomes in the hybrid cells were determined by karyotyping and by the analysis of human chromosome-specific isozyme markers^{12,14}. The phigf1 probe hybridized to three *Eco*RI fragments of 8.0, 6.6 and 4.3 kilobases (kb) from human DNA (Fig. 1a, lane 7) and three fragments of 6.7, 2.1 and 1.9 kb from mouse (Fig. 1a, lane 8). The isolation and characterization of human IGF-I and IGF-II indicates that they are single-copy genes (N. Fong and G.I.B., unpublished). The presence of human bands which hybridize with phigf1 correlated only with the presence of human chromosome 12 in an analysis of 31 hybrids (Table 1, Fig. 1a). No other chromosome or chromosome-specific isozyme marker co-segregated with phigf1 (Table 1). Figure 1a shows hybridization to DNA from hybrids containing human chromosome 12 (lanes 1–3); lanes 4–6 show hybridization to DNA from hybrids lacking this chromosome. The results demonstrate that the gene coding for human IGF-I is localized on chromosome 12. Analysis of human IGF-I in DNA from 13 unrelated individuals revealed a restriction fragment length polymorphism (RFLP) after digestion with *Pvu*II (Fig. 1b) or *Hind*III (data not shown). Hybridization of phigf1 to *Pvu*II-digested DNA yielded three bands of 8.2, 5.0 and 2.8 kb (Fig. 1b, lanes 1–3). The heterozygous pattern yielded an additional band of 5.4 kb (Fig. 1b, lanes 4 and 5). Preliminary data indicate that the polymorphic *Pvu*II and *Hind*III sites are linked.

Human IGF-II was mapped in the same manner as IGF-I using the phigf2 probe. After a *Hind*III digest of cellular DNA, phigf2 hybridized to three bands of 17, 8.5 and 4.9 kb in the human (Fig. 1c, lane 7) and a single band of 11 kb in the mouse (Fig. 1c, lane 8). Hybridization of the phigf2 probe with DNA from 31 cell hybrids revealed that the human bands correlated with the presence of human chromosome 11 (Table 1, Fig. 1c). No other chromosome or isozyme marker co-segregated with phigf2. Figure 1c shows a filter containing DNA from chromosome 11-positive (lanes 1–6) and negative (lanes 2–5) hybrids.

The localization of IGF-II on chromosome 11 was accomplished using somatic cell hybrids retaining different regions of chromosome 11. The cell hybrid XER7 (Table 1) lacked a normal chromosome 11 and 90% of the cells retained the recombinant chromosome which included the 11p11–11qter region¹⁸. As human IGF-II was absent from this hybrid, it must lie in the 11p11–11qter region. Hybrid EXR5CSAz (Table 1) retained the 11q13–11qter region but not an intact chromosome 11 (ref. 18).

Table 1 Segregation of cloned IGF DNA with human chromosomes in somatic cell hybrids

	IGF-I	IGF-II	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	TL*
Wil 1	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	+	-	-	-	+	-	+	
Wil 2	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	+	-	+	-	-	-	+	-	+	
Wil 5	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	+	-	+	
Wil 6	-	+	-	+	-	+	+	+	+	+	-	+	+	-	-	+	-	-	+	-	+	+	+	-	+	
Wil 7	-	+	-	+	+	-	+	+	-	+	-	+	+	-	+	+	-	-	+	+	-	-	+	-	+	
Wil 8	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	
Wil 8X	+	+	-	-	+	+	+	-	+	+	-	+	+	+	-	+	-	-	+	+	+	+	+	-	+	
Wil 13	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	
Wil 14	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	
Wil 15	+	+	-	+	+	+	-	-	+	-	-	+	+	+	+	+	-	-	+	+	-	+	+	-	+	
Rew 5	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	-	+	+	+	
Rew 7	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	
Rew 8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	
Rew 10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	
Rew 11	+	+	-	-	-	+	-	-	-	-	-	-	+	+	+	-	+	-	-	-	-	+	+	-	+	
TSL 1	-	+	-	-	+	+	-	-	-	-	-	+	+	-	+	+	-	+	+	+	-	+	-	-	-	
TSL 2	+	-	-	+	-	-	+	+	-	-	-	+	-	+	-	-	-	-	-	+	-	+	+	-	+	17/3
JSR 17S	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	7/9
JSR 29	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
JVR 22	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	2/1
JWR 22h	+	+	-	-	-	+	-	+	-	-	+	+	+	+	-	+	+	-	+	+	-	+	+	-	-	2/1
JWR 26C	+	+	-	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	-	+	-	+	+	-	+	1p ⁻
XTR 2	+	-	-	-	-	+	-	+	-	-	+	+	+	+	-	+	+	-	+	+	-	+	+	-	-	3/X
XTR 22	-	+	-	+	-	+	+	+	-	+	-	+	+	+	-	+	+	-	+	+	+	+	+	+	-	X/3
XTR3BSAgB	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	3/X, 10q ⁻
ICL 15	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	+	+	-	-	
MH 21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	
ATR 13	+	-	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	-	-	-	-	
REX 12	-	+	-	+	+	-	-	-	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	+	-	20/X
XER 7	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	+	11/X
EXR-5CSAz	+	-	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	-	X/11
% Discordancy† IGF-I			35	35	35	32	32	35	35	39	45	35	45	0	32	39	29	45	45	29	39	26	35	58	45	
% Discordancy† IGF-II			42	26	26	23	35	32	32	42	48	26	0	42	29	23	39	35	42	32	35	29	39	42	39	

Chromosome-specific enzyme markers also were determined on the same cell passage for these hybrids, confirming the chromosome analysis. Presence (+) or absence (-) of a human chromosome is indicated. Human female parental cells were used, eliminating the Y chromosome. IGF-I and IGF-II were determined by scoring the presence (+) or absence (-) of the DNA fragments containing the respective gene sequences.

* Well-defined human translocation chromosomes are retained in some hybrids; these are referenced in the text¹⁸.

† Concordant hybrids are those that have either both retained or lost IGF-I or IGF-II and a specific human chromosome. Discordant hybrids are those that either retained the genes but not a specific chromosome, or the reverse. % Discordancy indicates the degree of discordant segregation for a marker and a chromosome. Lack of discordancy demonstrates chromosome assignment.

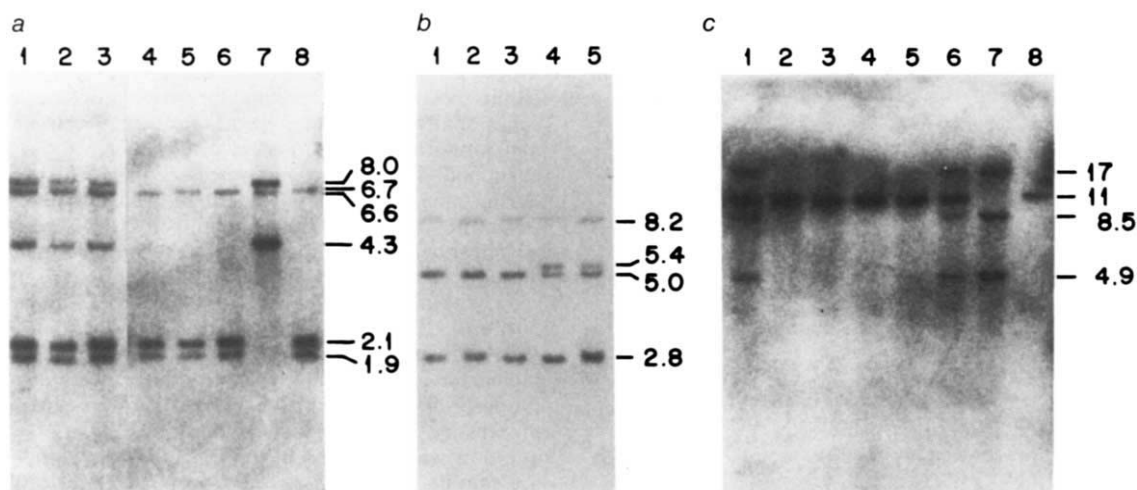


Fig. 1 Southern hybridization of cDNA probes of phigf1 and phigf2 to restriction digests of DNA from human, mouse and somatic cell hybrids. The lengths of the DNA bands are shown at the right of each figure as determined by size markers. *a*, Hybridization of IGF-I to cell hybrid DNA digested with *Eco*RI. Lanes 1-3, hybrids positive for chromosome 12; lanes 4-6, hybrids negative for chromosome 12; lane 7, HeLa cells; lane 8, mouse LMTK⁻ cells. *b*, Hybridization of IGF-I to leukocyte DNA digested with *Pvu*II from five individuals. Lanes 1-3, normal restriction pattern; lanes 4, 5, heterozygous pattern with an additional band at 5.4 kb. A similar heterozygous pattern was seen in these same individuals using *Hind*III. *c*, Hybridization of IGF-II to cell hybrid DNA digested with *Hind*III. Lanes 1 and 6, hybrids positive for chromosome 11; lanes 2-5, hybrids negative for chromosome 11; lane 7, HeLa cells; lane 8, mouse LMTK⁻ cells.

This hybrid was also negative for IGF-II, supporting the results for XER7. These data suggest that IGF-II is localized within the 11pter-11p11 region. Interestingly, the structurally related insulin gene is also localized on the short arm of human chromosome 11 at p15 (ref. 19).

Apart from the insulin and IGF-II genes, the short arm of chromosome 11 also encodes the c-Ha-ras1 oncogene and the lactate dehydrogenase A (LDHA) gene¹⁹. The short arm of chromosome 12 encodes the structurally related genes c-Ki-ras2 and the lactate dehydrogenase B (LDHB) genes¹⁹. In the mouse these genes are in conserved groups on chromosomes 7 and 6, respectively²⁰. These comparative gene mapping data suggest an apparent common ancestry for these sites.

Deficiencies in IGFs are linked to shortness of stature in humans⁷, while their excess is associated with acromegalia²¹. Examination of the structure of these genes in individuals of short stature could indicate the role of IGFs in the aetiology of dwarfism. The recent observations of homologies between the sequences of the B-subunit of platelet-derived growth factor and the protein encoded by the oncogene v-sis²², and the tyrosine kinase domain of the epidermal growth factor receptor and the v-erb-B gene product²³ indicate that cellular transformation could result from abnormal expression of a growth factor or its receptor. Moreover, the synthesis of growth factors by some tumours suggests that they may function as autocrine regulators in tumour growth¹¹. To date, neither IGF-I and IGF-II nor their receptors have been implicated in the transformation process, although the IGF-I receptor, like the EGF receptor and several oncogenes, has an associated tyrosine kinase activity²⁴. The assignment of the IGF genes to human chromosomes 11 and 12 suggests that tumours or hereditary syndromes containing rearrangements and amplification of regions of these chromosomes should be evaluated for abnormal expression of these growth factors. For example, duplication of the p13 to pter region of chromosome 11 has been demonstrated in the Beckwith-Wiedemann syndrome²⁵, which results in larger birth size, macroglossia and hyperplastic visceromegaly. The features may result from overproduction of IGF-II. The fine structure physical mapping of these genes will allow an assessment of the relationships of IGFs to proto-oncogenes, and to structurally defective sites, on the short arms of chromosomes 11 and 12. The screening of families for the polymorphisms associated with IGF-I will allow us to search for a relation between hereditary defects and this gene, and for close linkage relationships with other genes on this chromosome.

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Segmentation in the vertebrate nervous system

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Although there is good evidence that growing axons can be guided by specific cues during the development of the vertebrate peripheral nervous system¹, little is known about the cellular mechanisms involved. We describe here an example where axons make a clear choice between two neighbouring groups of cells. Zinc iodide-osmium tetroxide staining of chick embryos reveals that motor and sensory axons grow from the neural tube region through the anterior (rostral) half of each successive somite. 180° antero-posterior rotation of a portion of the neural tube relative to the somites does not alter this relationship, showing that neural segmentation is not intrinsic to the neural tube. Furthermore, if the somitic mesoderm is rotated 180° about an antero-posterior axis, before somite segmentation, axons grow through the posterior (original anterior) half of each somite. Some difference therefore exists between anterior and posterior cells of the somite, undisturbed by rotation, which determines the position of axon outgrowth. It is widespread among the various vertebrate classes.

In the chick embryo, the outgrowth of both motor and sensory axons occurs in an antero-posterior (rostro-caudal) sequence, those opposite the wing bud appearing between stages 16 and 17 (ref. 2) of embryonic development. By this stage the somite, whose cells growth cones first encounter, has developed into the outer dermatome-myotome (respectively prospective dermis and skeletal muscle) and inner sclerotome (prospective vertebral column). Motor axons leave the neural tube and grow laterally through the sclerotome towards the myotome (Fig. 1a); sensory axons appear when the dorsal root ganglion cells, within the sclerotome, send processes both peripherally (to join the motor axons) and centrally towards the dorsal portion of the neural tube. As originally described by Tello³, and visible in our whole-mount preparations, motor axons precede sensory axons when growing laterally through the sclerotome.

From the start, motor axons grow exclusively through the anterior half of their respective somite (Fig. 1b). Because growth cones and their filopodia are visible with the zinc iodide-osmium tetroxide stain (Fig. 1c), any axons present in the posterior half are unlikely to have been missed because of their failure to stain. In each of 73 somites examined from 10 stage-17 embryos, motor axonal growth was confined to the anterior half of the somite. In the case of sensory axons, however, processes can be seen occasionally to extend a short distance within the posterior half-somite; 6 such processes were seen in the 73 somites examined. These posterior processes have not been observed during later stages of development, and it is likely that they represent outgrowths from neural crest cells residing in the posterior half-sclerotome which later migrate or degenerate. All motor axons, therefore, and the great majority of sensory axons, grow within the anterior half-somite, specifically its sclerotome portion.

Several questions arise from this observation. First, is the segmented growth of motor axons determined by some intrinsic property of the neural tube, or is it, as the segmented growth of sensory axons suggests, determined by the somite? This question was answered by rotating the neural tube relative to the somitic mesoderm so the neural tube previously opposite the anterior half of the somite became positioned opposite the posterior half. Despite such displacement, in each of 11 such experiments axonal growth remained confined to the anterior half of the somite (Fig. 2). The neural tube is not, therefore, intrinsically segmented with respect to motor axon outgrowth (see also refs 4, 5).

To test whether segmentation instead arises from some property of the somites, the somitic mesoderm was rotated

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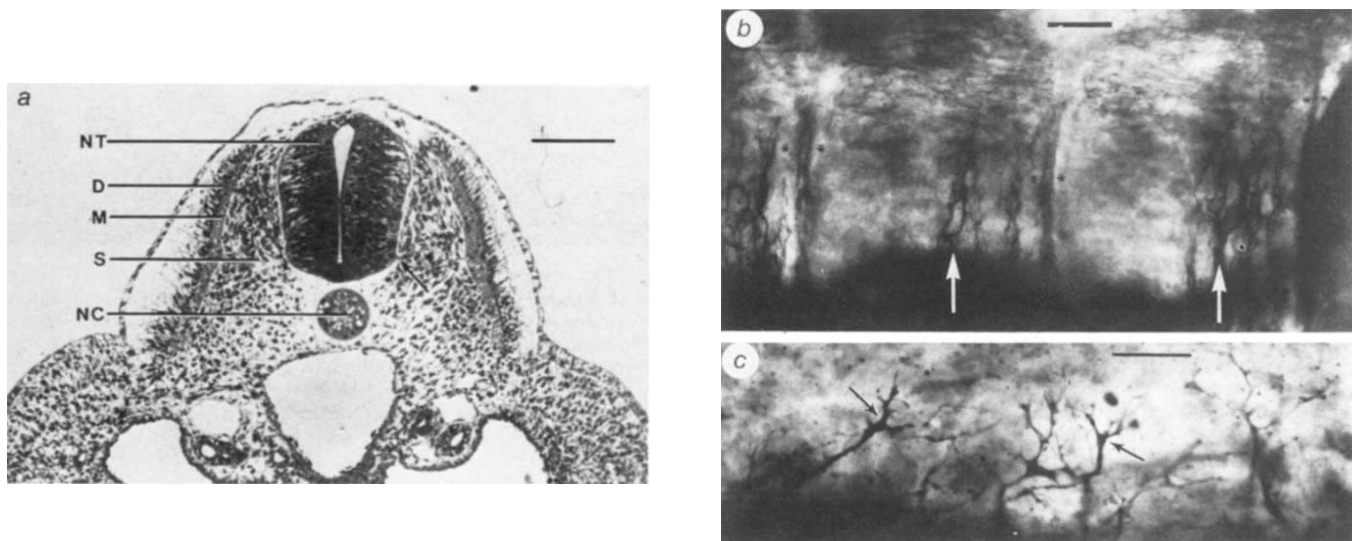


Fig. 1 *a*, Transverse section of a late stage-16 chick embryo, at the level of the wing somites, stained with toluidine blue. The embryo was fixed by immersion in buffered 2.5% glutaraldehyde, plastic-embedded and sectioned at 2 μm . By the stage of motor axon outgrowth (arrow), the somite has developed into dermatome-myotome (D, M) and sclerotome (S). NT, neural tube; NC, notochord. Scale bar, 100 μm . *b*, Late stage-16 embryo, wing region, stained with zinc iodide-osmium tetroxide. The staining procedure was a modification of the method described by Akert and Sandri²³. The embryos were pinned out on Sylgard dishes and bisected along the antero-posterior axis into left and right halves. They were then immersed in a freshly prepared mixture of 6 ml ZnI_2 /1.75 ml of 2% OsO_4 , to which had been added a small drop of a concentrated KI_3 solution. The ZnI_2 solution was made by combining 5 g iodine with 15 g powdered zinc in 200 ml water. They were then incubated at 55 $^\circ\text{C}$ for 100–105 min, washed with distilled water, dehydrated in alcohols, cleared in xylene and whole-mounted in Permount. Motor axons are seen in the anterior (right in the figure) halves of two somites, having emerged from the neural tube (arrows). The somite borders are enclosed by asterisks. Sensory axons were visible in another (more dorsal) plane of focus. Scale bar, 50 μm . *c*, Whole mount of a stage-21 embryo, wing region, stained with zinc iodide-osmium tetroxide. The end of a spinal nerve is shown, to demonstrate growth cones and their filopodia (arrows). Scale bar, 20 μm .

antero-posteriorly through 180 $^\circ$, leaving the neural tube undisturbed. Strips of segmental plate mesoderm, three to four prospective somites in length, were removed from donor embryos and implanted in host embryos, opposite the wing bud, after rotation (Fig. 3). Eight such operations were performed, in six of which the medio-lateral axis was also reversed. In all eight cases, axonal growth was now confined to the posterior (original anterior) halves of the grafted somites. Both anterior and posterior to the graft, axonal growth was, as expected, through the anterior halves of the host somites. In five control experiments where the segmental plate mesoderm was removed and replaced with the normal orientation, and in two where only the medio-lateral axis of the segmental plate was reversed, axons were positioned as in unoperated embryos. These experiments show that neural segmentation results from the somites. They also show that the anterior–posterior difference must be determined at or before somite segmentation from the segmental plate, and that the embryonic axis is unable to regulate for its positional disturbance. Furthermore, rotation about the medio-lateral axis is without effect on the segmentation of neural outgrowth.

What properties of the somite might restrict axonal growth to its anterior half? As axons first encounter sclerotome cells (Fig. 1*a*), there is presumably a difference between those cells in the anterior half-sclerotome and those in the posterior half-sclerotome; anterior cells permit axonal growth and/or posterior cells inhibit it. One possibility would be that motor axons emerge simultaneously opposite both sclerotome halves, but can only grow out through anterior sclerotome. We might then expect to see axons in the posterior region growing along the surface of the neural tube towards the anterior half-sclerotome. However, we do not (Fig. 4*a*). The first axons emerge only from neural tube opposite the anterior half-sclerotomes; later axons, though, do emerge opposite the posterior half-sclerotomes and then turn towards the anterior half-sclerotome on either side (Fig. 4*b*). Chick motor axons grow laterally from their cell bodies so as to leave the neural tube at the same antero-posterior level as that at which they arise⁶. Therefore, and contrary to Tello's

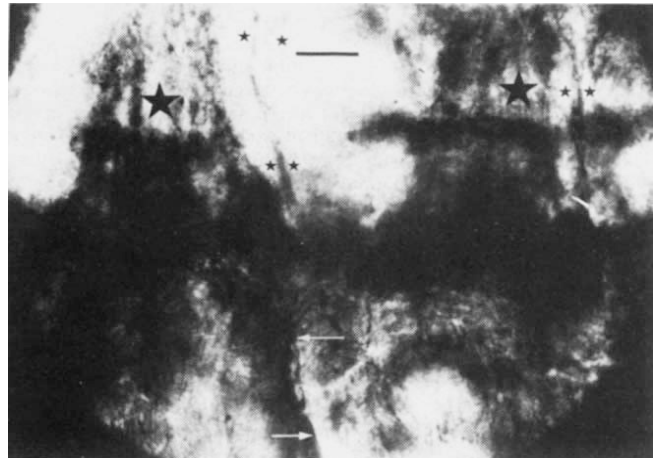


Fig. 2 Neural tube rotation: 11 host embryos were incubated at 38 $^\circ\text{C}$ to stage 11–13. Donor embryos were incubated to the same developmental stage and then pinned out in Sylgard dishes containing 0.1% trypsin (Difco) in calcium- and magnesium-free Tyrode's solution at room temperature. Within 3 min the neural tube could be removed, free of ectoderm, endoderm and notochord. Lengths of neural tube opposite two, three or four somites were cut from the presumptive wing region and their dorsal–anterior portions marked with carmine particles. Neural tube of the same length and position was removed from host embryos *in ovo* with fine tungsten needles, leaving the notochord and somites intact. The donor grafts were then implanted in the host embryos, after 180 $^\circ$ reversal about the antero-posterior axis. In this way, neural tube previously opposite an anterior half-somite came to lie opposite the posterior half. After 2 days' further incubation *in ovo*, the embryos were stained and examined as described for Fig. 1*b*. In the example shown, the junction between donor and host neural tube is arrowed: axons (large asterisks) grow through anterior (right) halves of somites from both donor (left) and host (right) neural tubes. Small asterisks enclose somite borders. Scale bar, 50 μm .



Fig. 3 Rotation of somitic mesoderm; technique as Fig. 2 legend. Lengths of segmental plate mesoderm, two to four prospective somites in length, were cut from the wing region of donor embryos and marked with carmine at one end. Similar lengths of segmental plate mesoderm were removed, together with overlying ectoderm, from the wing region of the same or opposite side of host embryos, leaving neural tube and endoderm intact. The grafts were then implanted in the hosts after 180° reversal about the antero-posterior axis or both antero-posterior and medio-lateral axes. Following 2 days' further incubation *in ovo*, the embryos were examined as for Fig. 1b. In the photomontage the neural tube is inferior. The dashed line separates the host region (left, posterior) from the graft region (right, anterior). Arrows mark the somite borders. In the graft region axons are now immediately anterior to the somite borders. Scale bar, 50 μ m.

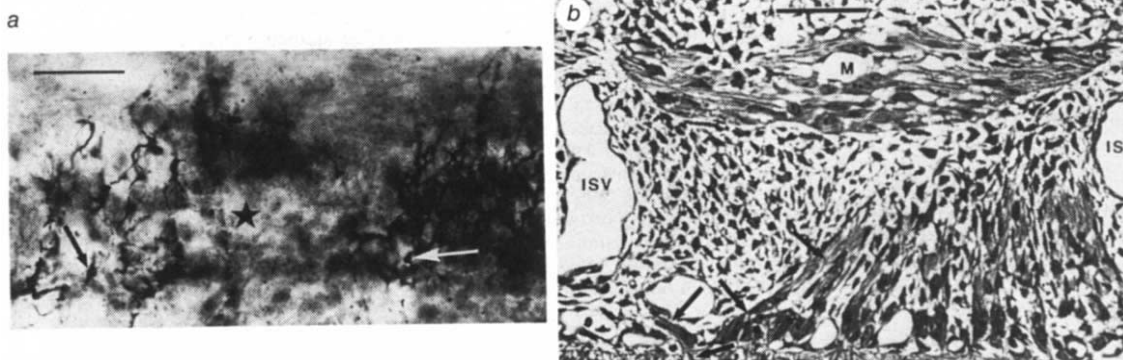


Fig. 4 *a*, Whole mount of neural tube and sclerotome of a late stage-16 embryo, wing region, stained with zinc iodide-osmium tetroxide. After staining, the neural tube was bisected along its long axis and most of the somitic mesoderm cut off laterally. The medial portions of two sclerotomes are left adjacent to the neural tube. Motor axons leave the ventral surface of the neural tube (arrows) and grow (initially dorsolaterally) in the anterior half of each sclerotome. No axons are seen near the surface of the neural tube opposite the posterior half of the sclerotome (asterisk), the same being true for adjacent planes of focus. Scale bar, 50 μ m. *b*, Longitudinal section of a stage-21 embryo, prepared as for Fig. 1a, stained with toluidine blue. Neural tube inferior (asterisks); anterior to the right. Axons emerging opposite posterior half-sclerotome (arrows) are seen, directed either anteriorly or posteriorly towards, respectively, the anterior half of the same somite or the anterior half of the adjacent (posterior) somite. The myotome (M) is seen straddling the intersegmental blood vessels (ISV). Scale bar, 50 μ m.

description³, the extension of motor axons occurs in a discontinuous punctuated manner along the neural tube, being first from cell bodies opposite the anterior half-sclerotomes. Only later do cells opposite the posterior half-sclerotomes extend axons; and these axons presumably fasciculate on the previous 'pioneer' outgrowths, after penetrating the basal lamina surrounding the neural tube. If axon extension is initiated as a consequence of some influence of the anterior half-sclerotome cells, this influence is presumably not an absolute requirement for axon extension, for prior destruction of the somitic mesoderm, at least at the stage of somite segmentation, does not prevent axon outgrowth⁵.

Since Remak's⁷ original observations on the development of the vertebrate column, there have been many further descriptive studies (see refs 8–10 for reviews). It has often been noted that the sclerotome subdivides into anterior and posterior regions of, respectively, lesser and greater cell densities, a difference which arises after the first axonal outgrowths (unpublished observations). Remak also saw that the spinal nerve and ganglion develop in the anterior half-sclerotome. Von Ebner¹¹ described a relatively cell-free space or fissure between anterior and posterior sclerotome halves in both snake and chick embryos, a feature which has been variously confirmed⁸ or refuted as a fixation artefact⁹. We confirm its existence in the chick embryo, finding it visible in the presence or absence of fixation. Axons have never been seen to cross this boundary

between anterior and posterior sclerotome halves, so it is likely that the fissure also represents the boundary between anterior and posterior portions of sclerotome with respect to axon growth.

Detwiler's⁴ classical experiments on neural segmentation in the axolotl showed that neural segmentation in this species results from mesodermal segmentation. Could, then, an antero-posterior subdivision of the sclerotome determine neural segmentation in all vertebrates? Certainly anterior-posterior sclerotome cell density differences have been described in all vertebrate classes^{7–9,12–17}, and the spinal nerve is seen in the anterior half-sclerotome. What is critical, however, is the timing of appearance of the sclerotome changes in relation to axon outgrowth. In the case of teleost¹⁸ and elasmobranch¹⁹ fishes, and anuran amphibia^{20,21}, where studied, the first motor axons grow out at a stage when there may be few or no sclerotome cells intervening between neural tube and myotome. In these conditions the myotome cells alone may determine segmentation. Nevertheless, sclerotome cells have been described in association with axons^{19,21}, and such myotomal determination of neural segmentation does not easily explain the coincident segmentation of sensory axons. Furthermore, in at least one elasmobranch fish, *Scylium catulus*, there is a sclerotome-filled space, several cell diameters wide, between neural tube and myotome at the stage that ventral root axons first appear (ref. 22 and our own unpublished observations). Like *S. catulus* and

the chick, in apodan amphibia¹³, reptiles¹⁵, other birds¹⁶ and mammals¹⁷, motor and sensory axons probably first grow out at a stage when sclerotome cells have accumulated between neural tube and myotome (Fig. 1a). Therefore, it is presumably towards this population of cells that we should look for an explanation of neural segmentation in these vertebrates. The experiments described here have shown that both motor and sensory axons preferentially associate with cells of the anterior half-sclerotome. How anterior and posterior cells differ in terms of cell lineage and molecular properties remains to be determined.

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Immune sera recognize on erythrocytes a *Plasmodium falciparum* antigen composed of repeated amino acid sequences

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Protective immune responses against the asexual stages of the human malaria parasite, *Plasmodium falciparum*, are most probably directed against exposed antigenic determinants on the surface of the free merozoite^{1–3} or the infected red blood cell⁴, and therefore antigens in these locations are candidates for testing as components of a defined molecular vaccine. To facilitate the search for such antigens, we recently developed a method for the expression of *P. falciparum* proteins in *Escherichia coli* as fused polypeptides⁵. Many clones producing antigens were detected by screening with immune human sera^{6,7}. We show here that antibodies against the fused polypeptide expressed by one such clone react with a *P. falciparum* protein that is synthesized late in schizogony and is later present on the surface of the ring-infected erythrocyte. The protein is composed of repeating subunits of 8, 4 and 3 amino acids and is present in all isolates of *P. falciparum* examined.

The construction of a cDNA library of *P. falciparum* in the vector λ gt11-Amp3 has been described previously⁸. cDNA was inserted into the unique *EcoRI* site near the C-terminus of β -galactosidase, resulting in expression of fused polypeptides in *E. coli*. Clones expressing fused polypeptides that correspond

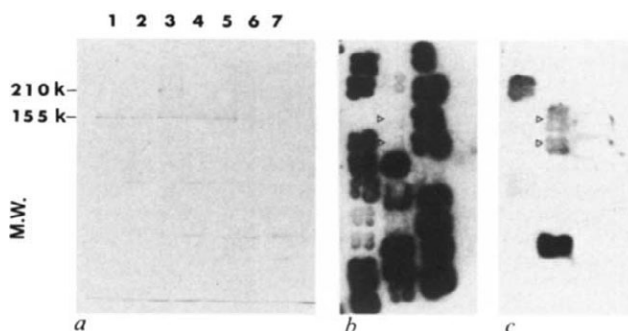


Fig. 1 Identification of the native protein corresponding to Ag13. *a*, One-dimensional SDS-polyacrylamide gels of immunoprecipitates *b*, *c*, Hybridization of stage-specific *P. falciparum* cDNA to antigen-positive clones.

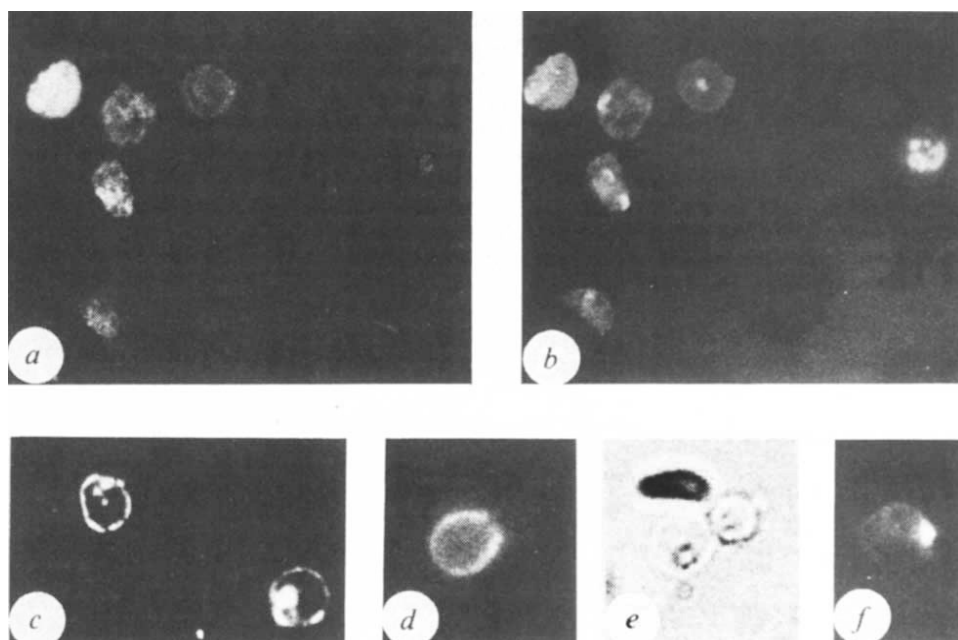
Methods: *a*, Parasites of the FC27 (ref. 18) line were grown in asynchronous culture *in vitro* according to the method of Trager and Jensen¹⁹. At a parasitaemia of ~3% the parasites were transferred to a methionine-free medium supplemented with ³⁵S-methionine for 18 h. Parasites were collected by centrifugation and lysed in 0.5% Triton X-100, 150 mM NaCl, 10 mM EDTA and 5 mM Tris pH 8. Aliquots of labelled parasite lysate were reacted with various sera. Formalin-fixed *Staphylococcus aureus* was used as a solid-phase reagent. Immunoprecipitates were prepared and analysed by one-dimensional SDS-polyacrylamide gel electrophoresis as described previously²⁰. Gels were fluorographed. The antisera were prepared by injecting mice with lysates²¹ of Ag13 or five independent antigen-positive clones (Ag24,28,32,46,47) producing fragments of the same molecule^{6,22}. These clones were identified by cross-hybridization with the Ag13 cDNA⁶ and by positive signal on the colony immunoassay with anti-Ag13 antibodies²². All sera recognized a 155K MW molecule but some also recognized a 210K MW molecule. Lane 1, anti-Ag28; lane 2, anti-Ag13; lane 3, anti-Ag24; lane 4, anti-Ag46; lane 5, anti-Ag47; lane 6, anti-Ag32; lane 7, anti- λ Amp3 vector. *b*, *c*, Parasites of the FC27 line¹⁸ were synchronized twice by treatment with 5% sorbitol according to the method of Lambros and Vanderberg²³ as modified by Freeman and Holder²⁴. The parasites were cultured *in vitro*¹⁸ for one cycle of growth and merozoites collected according to the method of Freeman and Holder²⁵. The merozoite preparation was stained with 10% Giemsa and examined under light microscopy and the degree of contamination by other life cycle forms was less than 1 infected cell per 1,000 merozoites. In a separate synchronization experiment parasites were collected at the transition point between mature trophozoites and early schizonts. No schizonts with greater than four nuclei were seen by light microscopy and trophozoites constituted over 75% of the parasites present. The parasite preparations were solubilized in 6 M guanidine hydrochloride in 0.4 M sodium acetate pH 5.2 and RNA was prepared as described elsewhere⁵. Aliquots of total RNA from the two preparations were transcribed into labelled cDNA and 3×10^6 c.p.m. per ml of each were hybridized to prepared nitrocellulose filters²⁶. The nitrocellulose filter was a replica of an array of antigen-positive clones including Ag13 sequences (arrowed). In *b* the array was reacted with cDNA prepared from the trophozoite plus early schizont preparation while in *c* the array was reacted with cDNA from free merozoites. The Ag13 sequences are strongly recognized by the merozoite cDNA preparation.

to natural antigens were detected by an *in situ* immunoassay using immune human sera. One clone, designated Ag13, produced a large fused polypeptide with a molecular weight (MW) of 156,000 (156 K) which was presumably composed of 116K of β -galactosidase sequence and 40K of *P. falciparum* sequence⁵. Immunoblot analyses using anti- β -galactosidase antibodies and anti-*P. falciparum* antibodies confirmed that the fused polypeptide contained antigenic determinants recognized by both sera^{5,7}. Mice and rabbits were immunized with lysates of induced bacteria or with the fused polypeptide purified as described previously⁹.

The *P. falciparum* antigen corresponding to the Ag13 fragment was identified as an acidic protein of 155K MW, by immunoprecipitation of biosynthetically-labelled asynchronous

Fig. 2 Indirect immunofluorescence of *P. falciparum* reacted with anti-Ag13 antibodies. The same field was examined for fluorescein fluorescence only (a) or for fluorescein and propidium fluorescence (b). c, Combined fluorescein and propidium fluorescence of two ring-infected erythrocytes. The fluorescence in the centre of the erythrocyte results from the reaction of propidium iodide with the parasite nucleus.

Methods: These blood films were prepared from asynchronous cultures of the FC27 isolate of *P. falciparum* and fixed by immersion in 90% acetone, 10% methanol. The parasites were then reacted for 30 min at room temperature with a 1:50 dilution of serum from mice immunized with Ag13. After washing in mouse tonicity phosphate-buffered saline, fluorescein-conjugated sheep anti-mouse IgG antiserum was added. Slides were washed in mouse tonicity phosphate-buffered saline and briefly reacted with 0.0005% propidium iodide for visualization of parasite nuclei. The slides were immersed in glycerol and examined under UV light. Non-fixed cells were also examined. *In vitro* cultures of FC27 were reacted with rabbit antibodies to the purified fused polypeptide from Ag13 for 30 min at 4 °C. After washing in phosphate-buffered saline, fluorescein-conjugated goat anti-rabbit IgG antiserum was added. The cells were washed and examined under UV light. The same field was viewed under UV (d) or visible light (e). The fluorescein staining cell is non-pigmented. f, Apparent capping of the antigen on the surface of an infected erythrocyte.



cultures of the FCQ27/PNG(FC27) isolate of *P. falciparum* (Fig. 1a). Some antisera, especially from animals that had received multiple boosts with antigen, contained antibodies directed against a second protein of 210K (Fig. 1a, lanes 3, 5). Immunoblotting experiments of parasite antigens from synchronized cultures indicated that the 210K MW protein was detectable only in the schizont while the 155K MW protein predominated in the ring stage (data not shown). It is not clear whether this indicates a precursor-product relationship between the two proteins or that they are antigenically cross-reactive distinct gene products.

The location of the 155K protein was determined by indirect immunofluorescence studies against both fixed and unfixed parasitized cells of the FC27 isolate. When anti-Ag13 antibodies were reacted with fixed cells, fluorescence was associated with a small proportion of the erythrocytes (Fig. 2a). Fluorescence was visible over the whole surface of these erythrocytes or as a rim around the cell (Fig. 2a, c). Double staining with propidium iodide showed that the fluorescent erythrocytes contained a single parasite nucleus, that is, they were infected with ring forms (Fig. 2b). All cells infected with ring forms appeared to have this antigen but mature trophozoites showed minimal fluorescence. However, fully mature schizonts were also reactive but the fluorescence was localized to the parasite and not on the erythrocyte surface. Small fluorescent bodies of a size consistent with merozoites were also seen. Fluorescence of ring-infected erythrocytes was seen when unfixed cells were used (Fig. 2d, f), demonstrating that this antigen is located on the surface of the erythrocyte. In some cases the fluorescence was not uniformly distributed over the cell surface (Fig. 2f). Because of its location, this antigen has been named the ring-infected erythrocyte surface antigen (RESA). Fluorescence studies on four isolates from Papua New Guinea (FC27, MAD143, MAD147 and MAD71), a Thai isolate (K1) and Ghanaian isolate (NF7) showed the presence of a protein immunologically cross-reactive with RESA on the surface of ring-infected erythrocytes of all isolates (data not shown) and the stage specificity of fluorescence and the molecular weight of RESA distinguish it from the merozoite surface polypeptide of 195K MW (ref. 3). The relationship of RESA to glycophorin-binding molecules¹⁰ of the same approximate molecular weight (that is 155K and

210K) has not been established. However, an antigen which may be equivalent has been detected on fixed ring-infected erythrocytes by immunofluorescence using immune human sera¹¹.

RNA extracted from cultures of asynchronous asexual blood stages contains RESA mRNA in low abundance, as labelled cDNA hybridizes to Ag13 very weakly (data not shown). This is also true for RNA extracted from trophozoite and early schizont stages (Fig. 1b). However, RESA mRNA is greatly enriched in merozoites relative to other stages of the asexual cycle (Fig. 1c).

The single cDNA insert of the clone Ag13 was purified and sequenced by the dideoxy method¹². The cDNA was 979 base pairs (bp) long and hybridization studies confirmed that it was derived from clone Ag13 (data not shown). The nucleotide sequence and derived amino acid sequence of a portion of the cDNA are shown in Fig. 3. The sequence contains a highly ordered region, composed of five exact repeats of a 24-bp sequence encoding a strongly acidic octapeptide, Glu-Glu-Asn-Val-Glu-His-Asp-Ala. Immediately 3' to this the glutamic acid, asparagine and valine codons are arranged in repeating 12-bp units interspersed at irregular intervals by a related 9-bp unit. The 12-bp unit, of which there are at least 25 exact repeats in the Ag13 cDNA, corresponds to half of the 24-bp repeat. All codons are exactly conserved except for third position variation in the valine codon, but this does not alter the encoded amino acid. Repetitive subunit structures, but of constant unit length, have been described previously in the S-antigen of *P. falciparum*⁹ and the surface coat of the *Plasmodium knowlesi* sporozoite¹³. The Ag13 sequence appears to be conserved between isolates (see below), in direct contrast to the S-antigen sequence, which was demonstrated to vary between isolates⁹.

The genomic organization of the gene encoding RESA was studied in three *P. falciparum* isolates, FC27 from Papua New Guinea, K1 from Thailand and NF7 from Ghana. DNA from each isolate was digested with restriction enzymes, size-fractionated and transferred to nitrocellulose. The filters were probed with ³²P-labelled Ag13 DNA (Fig. 4). At moderate stringency (2 × standard saline citrate, 65 °C) the probe hybridized to DNA in all tracks, with several bands of hybridization in each track (Fig. 4a). The genomic organization appeared largely to be

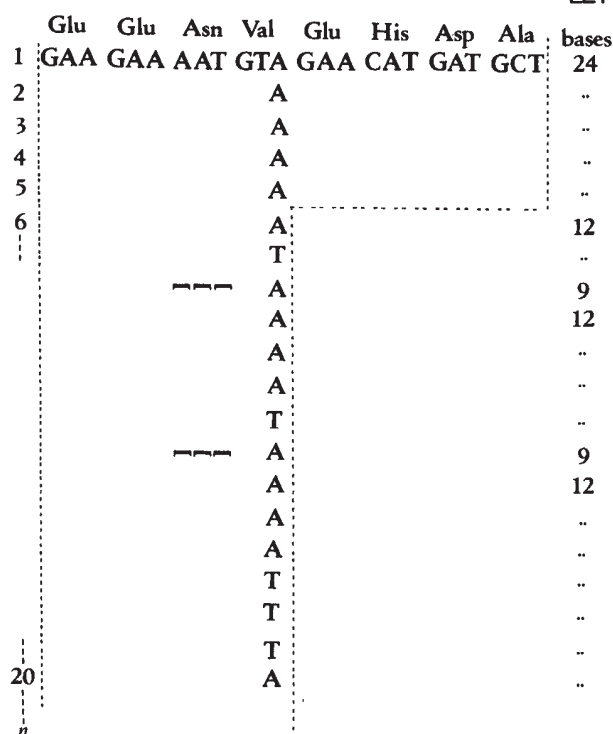


Fig. 3 Nucleotide sequence and predicted amino acid sequence of the Ag13 cDNA. The dideoxy chain termination method¹² was used for sequence determination. The Ag13 *Eco*RI cDNA fragment was subcloned into M13 mp9 (ref. 27) in both orientations and sequenced. The sequence shown starts 73 nucleotides 3' to the *Eco*RI site in the cDNA, but only the repetitive portion of the sequence is shown.

conserved in all three isolates. For example, there are common bands at 26, 11.8 and 6.4 kilobases (kb) in the *Eco*RI digests, with two unique bands in K1 of 7.5 and 9.8 kb. There was no hybridization to DNA from a human fibroblast line prepared and probed in similar conditions, demonstrating that RESA is a product of *P. falciparum* (data not shown). When the *P. falciparum* DNA was probed at high stringency (0.2× standard saline citrate, 65°C), only one band of hybridization was seen in each track (Fig. 4b) and this was identical for all isolates. This suggests that DNA sequences partially homologous to RESA occur in the genome. Whether these represent pseudogenes or functional genes that have diverged in sequence is not known.

The data here show that this 155K MW antigen is located on the surface of the ring-infected erythrocyte, and is also present in mature schizonts. The light microscopic studies cannot unequivocally locate RESA on the surface of the merozoite. However, a plausible model is that this protein is synthesized late in schizogony in the developing merozoite and after schizont rupture is carried to an uninfected erythrocyte where it is either stripped from the merozoite or actively secreted during invasion, remaining for some time on the erythrocyte surface. *De novo* synthesis of a 155K protein by the ring parasite, with subsequent transport to the erythrocyte membrane, seems unlikely in view of our failure to detect RESA on parasites within the ring-infected erythrocytes, and also our failure to detect specific synthesis of proteins of this molecular weight by ring parasites^{14,15}.

The exposed location of RESA on the surface of the infected erythrocyte presumably makes it vulnerable to immune attack. Anti-RESA antibodies are found in some sera from immune humans—indeed this was the basis for the initial detection of clone Ag13 (ref. 5). An immune response to the RESA could interfere with merozoite invasion or prematurely lyse recently infected cells, a previously unsuspected effector mechanism protecting against malarial infection. This antigen may therefore

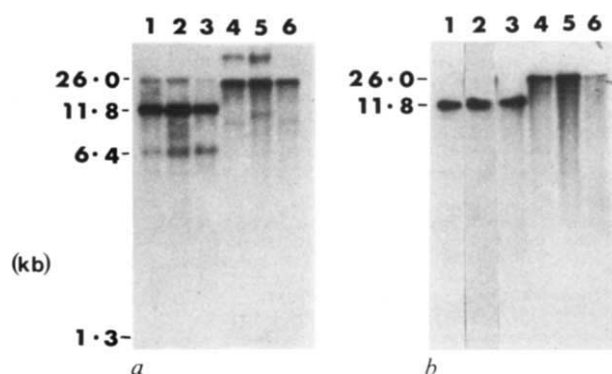


Fig. 4 Southern blot analysis of genomic DNA of *P. falciparum* by Ag13 cDNA under moderate and high stringency. *Eco*RI digest of FC27 DNA (lanes 1); K1 DNA (lanes 2); and NF7 DNA (lanes 3). *Hind*III digest of FC27 DNA (lanes 4) K1 DNA (lanes 5); and NF7 DNA (lanes 6). *P. falciparum* parasites from *in vitro* culture were collected and concentrated by lysis in 0.15% saponin solution⁵. The cell pellets were dissolved in 6 M guanidine hydrochloride, 0.4 M sodium acetate pH 5.2. The DNA was twice banded in CsCl gradients then dialysed against 10 mM Tris pH 8, 1 mM EDTA. Aliquots of 3 µg were incubated with either *Eco*RI or *Hind*III and the digestion products electrophoresed in a 1% agarose gel. The DNA was transferred to nitrocellulose²⁸ and the filters baked *in vacuo* then prehybridized in 5×SSC, 50% formamide, 50 µg ml⁻¹ salmon sperm DNA, 10 µg ml⁻¹ Poly(C) and 1×Denhardt's solution. Purified Ag16 cDNA was labelled by nick translation and hybridized to the filters at 10⁶ c.p.m. per ml at 42°C. The filters were washed in 2×SSC (a) or 0.2×SSC (b) at 65°C and autoradiographed.

have a role in protective vaccination. However, not all portions of the molecule may be equally immunogenic. We postulated that the extreme variability of repeat sequences in S-antigens was a mechanism to thwart strain-specific immunity^{9,16}. In contrast, the apparently conserved repetitive structures of the RESA may have a different role in immune evasion. Haptens densely packed on an antigenic molecule can mask an immune response to a different hapten on that same molecule¹⁷. This 'intramolecular competition' may enable the immunodominant repeat units of the RESA effectively to inhibit responses to other regions of the molecule. Consistent with this is the demonstration by Southern analysis that repeat units (Fig. 3) are confined to the 3' end of the RESA, entirely within the Ag13 cDNA, and the remaining portion of the molecule (~80%) contains no homologous sequences (data not shown). Expression of repetitive and non-repetitive segments of RESA in *E. coli* will allow this hypothesis to be tested directly.

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Monoclonal antibody production by receptor-mediated electrically induced cell fusion

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Fusion of myeloma cells and B lymphocytes to form hybridomas which produce monoclonal antibodies has been a major advance^{1–3}, but the poor efficiency and randomness of viral or polyethylene glycol fusion techniques generally gives poor yields of specific, high affinity antibodies. High voltage electrical fields with dielectrophoresis to ensure cell alignment can fuse a limited number of cells under direct microscopic examination^{4–9}, but it is not possible to identify B-cells destined to secrete relevant antibodies. However, B-cells express, on their surface, antigen receptor immunoglobulins of the same antigenic specificity as the secreted antibodies. Binding of antigen to surface immunoglobulins stimulates proliferation and differentiation of B-cells into plasma cells. Here we report the use of the selective, high affinity interaction of antigen with surface immunoglobulins on B-cells to facilitate a close adherence to myeloma cells. The antigen, covalently conjugated to avidin, binds to the surface immunoglobulins on B-cells. This B-cell–antigen–avidin complex binds to biotin covalently attached to the surface of myeloma cells. An intense electric field across a bulk cell suspension then produces selective fusion of cells in contact, that is, of myeloma cells with B-cells which make the appropriate antibody. We have used this technique with several antigens, and all resultant hybridomas secrete appropriate antibodies with very high affinity.

Antigen is linked to avidin via a small cross-linking molecule, 1,5-difluoro-2,4-dinitrobenzene (DFDNB). In this procedure it is essential that an adequate number of antigenic sites remain free to interact with the B-cells. Also, non-productive conjugation between avidin molecules or between antigen molecules must be minimized. Finally, it is necessary to preserve at least one biotin binding site on the avidin molecule. Therefore, the antigen–avidin conjugate is prepared using an immobilized, reversibly binding analogue of biotin, iminobiotin (Fig. 1a). Avidin is mixed with a large excess of iminobiotin coupled to Sepharose in order to minimize avidin–avidin cross-linking. Unbound avidin is then washed away. The bound avidin is reacted with 100-fold excess of DFDNB, which provides a large number of reactive sites for antigen on the avidin molecules. Following extensive washing to remove unbound DFDNB, antigen is added to the activated avidin bound to iminobiotin–

Sepharose. Free reactive sites on avidin are blocked with glycine. The antigen–avidin complex is eluted from iminobiotin with a pH 4 buffer. The conjugate now has at least one active biotin binding site.

The antigen–avidin conjugate is reacted with B-cells obtained from mice that have been immunized with the appropriate antigen. This reaction is conducted at 4 °C to prevent capping and internalization of the conjugate. The B-cells are then washed to remove unbound conjugate. Myeloma cells are reacted with an *N*-hydroxysuccinimide derivative of D-biotin. This procedure is gentle and myeloma cell viability is preserved. The myeloma cells are washed to remove unreacted biotin. The myeloma and B-cells are then incubated together at 4 °C to permit adhesion of the biotinylated myeloma cells with those B-cells having antigen–avidin conjugate bound to their surface immunoglobulins (Fig. 1b). A wide range of cell type ratios can be used.

The cells are suspended in sucrose and then exposed to a transient electric field generated by a high voltage pulse generator (Cober Electronics). To avoid severely damaging the cells, isotonic sucrose is used because of its low electrical conductance. A cell suspension is used in order to minimize random fusion of cells. To establish that the avidin–biotin conjugate is required for efficient cell fusion, we attempted 30 fusions using immunized or non-immunized mice under our standard experimental conditions but omitted the avidin–biotin conjugate procedure. Under these conditions the fusion frequency of cells is less than 10^{–8}. Typically, 0.15 ml of a suspension containing about 10⁷ myeloma cells and 10⁷ B-cells is exposed to four 5 µs pulses at 4 kV per cm. The instrument used to fuse cells has been described previously^{10,11}. Following fusion, cells are plated at about 10⁵ cells per well into plates seeded with about 10⁴ murine peritoneal macrophages per well. The medium contains aminopterin, which selects for hybrids¹². Wells with growing colonies are screened for antibody production.

In our initial studies, monoclonal antibodies were raised against rat lung angiotensin-converting-enzyme (ACE). We used two different immunization procedures in separate studies of antibody formation to ACE. In the first study (fusion A), a C57 BL/6 mouse received an intraperitoneal (i.p.) injection of crude rat lung membranes followed by a second injection four weeks later. After another two weeks, 2 µg of pure rat lung ACE (prepared by a modified method¹³) was injected intraperitoneally. Three days later the spleen was removed and the cells were fused. In a second experiment (fusion B), a C57 BL/6 mouse received two i.p. injections of pure ACE (2 µg) five days apart. Three days after the second immunization the spleen cells were fused.

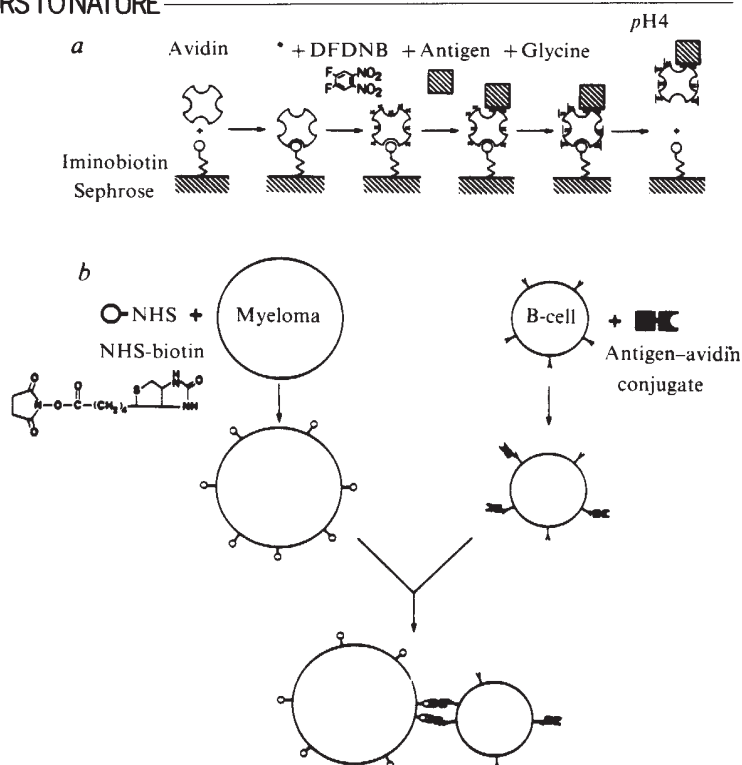
Half of the cells from fusion A were plated into 72 wells. A total of 31 wells produced growing hybrids after 6 weeks, and all wells gave evidence of antibody formation against ACE (Fig. 2). Half of the cells from fusion B were plated into 144 wells. Eleven wells showed cell growth and again all of these wells produced antibodies to ACE (Fig. 2). Examination of wells containing growing hybrids from fusion A and fusion B revealed only a single growing colony in each well. We cloned the hybridomas twice by limiting dilution¹⁴.

Subclass specific antisera were used to determine the immunoglobulin subtypes of 31 monoclonal antibodies. A spectrum of subtypes was generated: two were IgM, one was IgG1, one was IgG3, and the rest were either IgG2a or IgG2b. One antibody had λ light chains, the remainder reacted with anti-κ antiserum. Gel electrophoresis of six purified monoclonal antibodies revealed in all cases, a single light chain of molecular weight 20,000–25,000. For three of the antibodies, the heavy chain appeared as a doublet. These were all of the γ2b subtype. These results confirm the diversity found in the subclass typing experiments, and indicate that the antibodies examined were not derived from the same B-cell.

Monoclonal antibodies normally have lower antigen binding affinities than polyclonal antibodies raised against the same antigen. Since fusion of myeloma cells and lymphocytes in our study depended on antigen–antibody recognition, we reasoned

Fig. 1 Scheme for obtaining specific cell-cell adhesion: *a*, Preparation of antigen-avidin conjugate. *b*, Biotinylation of myeloma cells and specific adhesion to B-cells.

Methods: *a*, Iminobiotin-Sepharose (0.1 ml gel containing 1 μ mol iminobiotin), previously reacted with DFDNB and glycine, is incubated with avidin (10 nmol) for 2 h at 20 °C. The resin is washed with 10 ml 0.1 M Na borate buffer (pH 8.5) and 1 μ mol of DFDNB dissolved in 10 μ l methanol, then washed off after 10 min at 20 °C. Pure antigen (1 μ g) is reacted with the activated immobilized avidin for 12 h at 4 °C. After removal of unbound antigen, glycine (0.1 μ mol) is added to block the remaining reactive sites. The antigen-avidin conjugate is eluted from the resin with 0.2 ml 50 mM citrate-phosphate (pH 4). *b*, 10^7 – 10^8 Myeloma cells (P3 $\times$ 63Ag8.653) are washed with phosphate buffered saline, resuspended in 5 ml buffered saline and incubated with *N*-hydroxysuccinimide (NHS) linked to biotin (5×10^{-7} mol dissolved in 50 μ l dimethylformamide) for 1 h at 4 °C. Cells are extensively washed with cold Dulbecco's modified Eagle's medium (DMEM) containing deoxyribonuclease I (DNase I) (50μ g ml $^{-1}$) to remove unbound biotin. Spleen cells from an immunized mouse are incubated with the antigen-avidin conjugate in 5 ml DMEM for 4 h at 4 °C. Spleen cells are washed with cold DMEM containing DNase I to remove excess conjugate, mixed with biotinylated myeloma cells and centrifuged at 200 g for 5 min. The pellet is then incubated for 3 h at 4 °C and washed with DMEM containing biotin (10^{-6} mol ml $^{-1}$) and DNase I. The excess biotin is added to terminate the cross-linking process and prevent the formation of multicellular aggregates. Cells are finally resuspended in isosmotic sucrose containing 2 mM NaK phosphate (pH 7.2) adjusted to 290 mosmol and fused by four exposures to 4 kV cm $^{-1}$ for 5 μ s at 30 °C.



that hybridomas should produce high affinity antibodies even with spleen cells obtained during a primary response. Accordingly, we evaluated the affinity of our monoclonal antibodies for ACE. Their affinities (defined by the concentration of antibody required to bind 50% of added 125 I-labelled ACE) ranged from 10^{-8} M to 10^{-10} M. This high affinity was confirmed in kinetic experiments with antibodies A4 and A24, which bound to 125 I-labelled ACE with half lives of about 13 min; the reaction was completed in 30 min at 35 °C. Dissociation rates were obtained by incubating antibody bound to *Staphylococcus aureus* cells with excess 125 I-labelled ACE to equilibrium, removal of free 125 I-labelled ACE and addition of unlabelled ACE in 1,000-fold excess over bound 125 I-labelled ACE. The half life for dissociation at 35 °C was ~ 3 h for antibody A24 and about 8 h for antibody A4.

The specificity of the anti-ACE antibodies was established by a Western blot analysis (Fig. 3). Crude rat lung extract (containing ACE) was subjected to SDS-gel electrophoresis and transferred to a nitrocellulose membrane. Strips of the membrane were reacted with the various anti-ACE monoclonal antibodies and then stained with peroxidase conjugated to rabbit anti-mouse antibody. In all cases the anti-ACE antibodies reacted with only a single band which corresponded to authentic ACE. As ACE concentration in the rat lung extract was less than 0.1% of total protein, this experiment demonstrates the high specificity of all the monoclonal antibodies examined.

To assess the general applicability of this technique, we have also used it to develop monoclonal antibodies against enkephalin convertase, an enkephalin-forming carboxypeptidase¹⁵, and against the nine-amino acid peptide, bradykinin. A mouse received an i.p. injection of 2 μ g of enkephalin convertase mixed with complete Freund's adjuvant followed five days later by another injection of 2 μ g of enkephalin convertase alone. Three days later its spleen cells were linked to avidin-enkephalin convertase and fused with biotinylated myeloma cells. Four wells containing growing cells were obtained out of a total of 72 wells. All four wells had antibody activity against enkephalin convertase, as measured

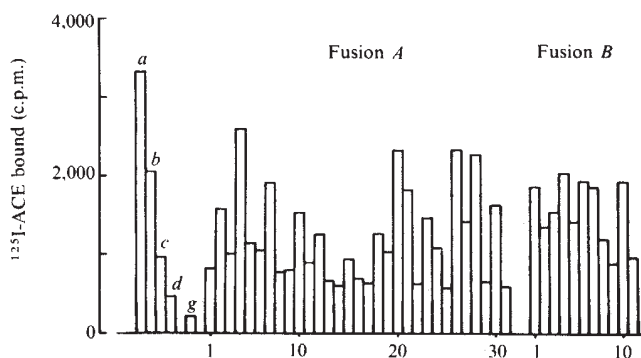


Fig. 2 Screening for anti-ACE activity. Supernatants (0.2 ml) from wells with growing hybridomas (31 from fusion A and 11 from fusion B) were incubated with 125 I-labelled ACE (2 pg consisting of about 50,000 c.p.m.) in buffer containing 50 mM Tris HCl, 0.2 M NaCl, 0.1% (w/v) Triton X-100 (pH 7.7) for 2 h at 35 °C. Rabbit anti-mouse IgG (2 μ g) was added and incubated for 1 h at 37 °C followed by addition of *S. aureus* cells (2.5 mg) and incubation for 1 h at 37 °C. The suspension was poured over Whatman GF/B filters, washed with 10 ml of buffer, and bound radioactivity determined. Positive controls consisted of a goat antiserum¹⁸, provided by Dr R. L. Soffer, raised against ACE and diluted as follows into normal goat serum: 3,000-fold (a), 10,000-fold (b), 30,000-fold (c), and 100,000-fold (d). Negative controls were normal mouse serum diluted from 1,000 to 100,000 (not shown) and a mouse IgG2a monoclonal antibody produced against a toxin from Russell viper venom (g). In either case, the amount of bound radioactivity was typically 300 c.p.m. with less than 5% standard deviation. Colonies developed between 15 days to 4 weeks after the fusion. Data shown here represents the anti-ACE activity in confluent wells. After 8 weeks, all wells containing growing cells, when assayed with 10 pg of 125 I-labelled ACE, had antibody activities at least five-fold greater than the negative control. Some clones had longer doubling times, up to 20–30 h. However, antibody concentrations were usually 1–5 μ g ml $^{-1}$ when cells were confluent.

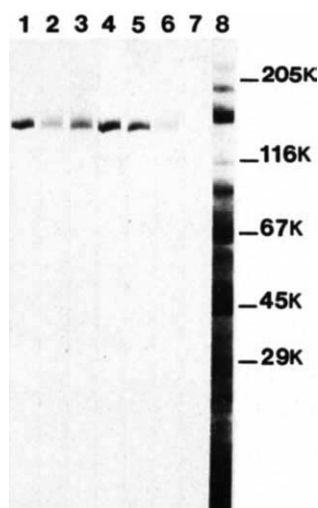


Fig. 3 Specificity of monoclonal antibodies staining crude rat lung extract transferred onto a nitrocellulose membrane after SDS-polyacrylamide gel electrophoresis. Rat lungs were homogenized, extracted (5 ml per g tissue) with 50 mM Tris HCl, 0.2 M NaCl and 1% (w/v) 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane-sulphonate (CHAPS) (pH 7.7), and dialysed to remove the detergent. The extract was electrophoresed on a 7.5% polyacrylamide gel, then electrophoretically transferred to a nitrocellulose membrane which was incubated with 20% calf serum for 1 h at 20 °C, and cut into strips. The strips were incubated for 4 h at 20 °C with purified antibodies from 6 cloned hybridomas—A4, A6, A23, A24, A26, and A28 (lanes 1–6, respectively) and washed 3 times with 20 mM Tris HCl, 0.2 M NaCl and 0.1% (v/v) Tween-20 (pH 7.7). A second antibody linked to peroxidase was used to visualize mouse antibody bound to the strips. A negative control (lane 7) was a mouse IgG2a monoclonal antibody raised against a toxin in Russell viper venom. The total protein present is demonstrated with a strip (lane 8) not incubated in serum but stained with 0.02% amido-black in methanol/acetic acid/water (50:10:40).

by depletion of soluble enzyme activity with antibody precipitated by *S. aureus* cells (manuscript in preparation). To develop antibodies to bradykinin, a mouse received an initial injection of 5–20 µg of bradykinin conjugated to human albumin mixed with complete Freund's adjuvant, followed five days later by an injection of the bradykinin–albumin conjugate alone. Spleen cells were fused three days later using a bradykinin–avidin conjugate. Seven wells (from a total of 120 wells) contained growing cells, and all demonstrated antibody activity to bradykinin as determined by radioimmunoassay using methionyl-lysyl-bradykinin labelled with ^{125}I (Bolton–Hunter) (manuscript in preparation).

Screening of these antibodies involved immunoprecipitation by *S. aureus* cells at 35 °C, in filtration or centrifugation assays. Under these conditions, immunocomplexes with affinities that are weaker than $K_D = 10^{-8}$ M dissociate too rapidly for detection. Similar methods have been widely used to study ligand binding to neurotransmitter receptors, and in these experiments, also, complexes with K_D values greater than 10^{-8} M are not detectable¹⁶. This indicates that the anti-bradykinin and anti-enkephalin convertase antibodies have high affinity for the antigens, with dissociation constants presumably less than 10^{-8} M.

To confirm that hybridomas formed by our technique result from antigen–antibody recognition, we performed the following experiment. A pure F(ab')₂ fragment of sheep antibody raised

against mouse IgM and IgG was covalently conjugated to avidin and used to elicit fusion between biotinylated myeloma cells and mouse spleen cells. One per cent of the spleen cells fused with myeloma cells and were selected in a medium containing hypoxanthine, aminopterin and thymidine. Most of these resultant hybridomas secreted mouse IgG (data not shown). No hybridomas were obtained when fusion was performed without a cell cross-linking conjugate.

Previously, electric field-induced cell fusion, with pronase treatment and dielectrophoresis to promote cell adhesion has not yielded monoclonal antibodies¹⁷. In initial experiments, with concanavalin A or pronase to elicit cell adhesion, we obtained fusion frequencies of 10^{-1} – 10^{-2} , which is 4,000–5,000 times the frequencies obtained with polyethylene glycol (manuscript in preparation). Furthermore, most of the hybridomas produced by electrical fusion, like other techniques, secreted mouse immunoglobulins. However, for the production of monoclonal antibodies, the resultant millions of growing colonies would pose a vast problem for screening. Accordingly, we deliberately reduced the frequency of cell fusion by diluting the cell suspensions and by avoiding agents which promote nonspecific cell adhesion. Instead, antigen–antibody recognition selects a small number of heterokaryons which secrete antibodies with high affinity for the antigen. The high affinity antibodies obtained to the enzymes ACE and enkephalin convertase and to the peptide, bradykinin, confirm the feasibility of this procedure and also indicate its general applicability.

The present technique provides a number of advantages over existing procedures. Very small amounts of antigen can be used. In conventional techniques for producing monoclonal antibodies, hyperimmunization with large amounts of antigen is usually attempted before fusion, in order to elicit a secondary response with antibodies of reasonably high affinity. In contrast, our procedure provides high affinity antibodies of mainly the IgG type from a primary response after immunization with minute quantities of antigen. This apparently occurs because of the selection by the avidin–antigen conjugate, of those few high affinity immunoglobulin secreting cells which are stimulated to proliferate during a primary response. The specificity of this cell fusion technique provides a great reduction in the number of growing hybridomas that must be screened for antibody production. With the present procedure, using the appropriate antigen–avidin conjugate, all hybridomas formed produce desired antibodies.

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Intron-less globin genes in the insect *Chironomus thummi thummi*

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The basic structural organization of the genes coding for vertebrate tetrameric haemoglobins and monomeric myoglobin has been strictly conserved throughout the past 600–800 million years of evolution¹. The occurrence of introns both in plant leghaemoglobin genes^{2–5} and in vertebrate globin genes suggests that an exon–intron configuration may have already existed in the ancestral globin gene from which all contemporary genes of the globin gene family evolved⁶. We report here the first analysis of invertebrate globin genes. Four closely linked globin genes have been isolated from a genomic library of the insect *Chironomus thummi thummi*, and characterized. Genes *A* and *B*, which are transcribed from opposite DNA strands, encode haemoglobin III (ref. 7), while genes *C* and *D* code for haemoglobin IV (ref. 8) and are also transcribed from different DNA strands. Surprisingly all four globin genes lack any intervening sequences. The genes have all the characteristics of productive globin genes and are expressed *in vivo*. The 5' end of the *C. t. thummi* globin genes encodes an amino-terminal signal peptide which is not present in mature haemoglobins. This is consistent with the observation that these haemoglobins represent secretory proteins, synthesized in the larval fat body and subsequently secreted into the haemolymph^{9,10}.

The aquatic larval stage of the midge *C. t. thummi* (Diptera) possesses at least 12 different haemoglobins which are found free in the haemolymph. The amino acid sequences of the individual haemoglobins have been determined^{17,8,11–20}. Five are monomers (components I, IA, III, IIIA, IV), six are homodimers (components II β , VI, VIIA, VIIB, VIII, IX) while component X has both monomeric and dimeric properties. The monomeric haemoglobins resemble myoglobin while the homodimeric haemoglobins emerged later in evolution²¹ and can be regarded as intermediates between monomeric and tetrameric haemoglobins.

To obtain a hybridization probe specific for larval globin sequences, ³²P-labelled cDNA was prepared from larval 8–14S poly(A)⁺ RNA enriched for globin mRNA (see legend to Fig. 1). In addition, double-stranded cDNA was prepared from 8–14S poly(A)⁺ RNA and cloned²² in pBR322 as previously described²³. Two independent cDNA clones were identified, by DNA sequencing, as coding for haemoglobin III (ref. 7) (data not shown). The genomic library used in this study was prepared by cloning partial *Eco*RI digests of *C. t. thummi* chromosomal DNA in the λ vector Charon 4A (H. Bäumlein, unpublished work). Approximately 280,000 recombinant phage of the genomic library were screened²⁴ for clones containing globin gene sequences. From 94 independently derived positive recombinant phage, 22 clones showing the strongest hybridization signals were plaque-purified and further investigated with respect to their DNA restriction pattern and coding potential. A detailed restriction map was established for one selected clone, CttG-1 (Fig. 1). The 15.5-kilobase (kb) CttG-1 insert DNA consists of seven *Hind*III restriction fragments, three of which hybridize to labelled cDNA prepared from larval 8–14S poly(A)⁺ RNA as well as to cloned globin III-specific cDNA. These are the 0.7-kb *Eco*RI–*Hind*III fragment adjacent to the left phage arm (marked *A* in Fig. 1c), the 3.7-kb *Hind*III fragment (*B*, *C*) and the 0.945-kb *Hind*III fragment (*D*), respectively. Fine restriction mapping of the 3.7-kb *Hind*III fragment revealed the presence of two separate globin gene sequences (*B*, *C*). Due to the similar amino acid sequences of haemoglobin III and haemoglobin IV (83% homology)⁸, genomic sequences hybridizing to globin III-specific cDNA are expected to code for either haemoglobin III or haemoglobin IV.

We have determined the entire nucleotide sequence of the 0.945-kb *Hind*III (*D*) fragment (Fig. 2) according to the

sequencing strategy outlined in Fig. 1. The deduced amino acid sequence corresponds exactly with that reported for haemoglobin IV⁸. Inspection of the gene sequence (Fig. 2) reveals that the conserved exon–intron configuration of vertebrate globin genes or of plant leghaemoglobin genes does not exist in this insect globin gene. The *C. t. thummi* globin IV gene lacks introns and thus represents a unique exception among all productive globin genes analysed so far. This globin gene has otherwise all the characteristics normally attributed to functional genes coding for proteins. The coding region of the gene is flanked by a 46 base pair (bp) 5' noncoding region and a 81-bp 3' noncoding region. Regulatory sequences present in most eukaryotic genes are also conserved in the *C. t. thummi* globin IV gene. In the 3' noncoding region, the T residue of the AATAAA sequence^{25,26} precedes the polyadenylation site by 22 nucleotides, while in the 5' flanking region the TATA box²⁷ is located 30 bp 5' to the capping site (Fig. 2). The absence of the CCAAT box²⁸ at position –70 to –80 is not unexpected as this sequence is also not present in the 5' flanking region of adult duck α -globin genes^{29,30} or the seal myoglobin gene¹.

At the 5' end of the *C. t. thummi* globin IV gene there is a region coding for an amino-terminal signal peptide. This is characteristic of secretory proteins^{31,32}. The amino acid sequence of the mature *C. t. thummi* globin IV starts with the sequence Leu-Thr-Ala (ref. 8). However, the only initiation codon AUG in the region spanning the cap site to codon 1 of the mature globin is located 45–43 bp 5' to codon 1 (Fig. 2) and allows^{33,34} in-phase reading of the adjacent signal peptide and the mature globin. We therefore assume that the primary translation product of *C. t. thummi* globin IV is a preglobin which carries an amino-terminal extension of 14 amino acids, 13 of which are hydrophobic. It is not clear whether the AUG triplet represents a translated initiation codon as, for instance, in the case of prealbumin and pretransferrin³⁵. The hydrophobic leader peptide is probably responsible for the vectorial transport of globin IV across the endoplasmic reticulum^{31,32}. These data are consistent with earlier studies suggesting that *C. t. thummi* haemoglobins are synthesized in the larval fat body and subsequently secreted into the haemolymph as free molecules⁹. Moreover, there is indirect evidence that *C. t. thummi* globins are synthesized as preoglobins¹⁰.

In many eukaryotic genes coding for secretory proteins, sequences specifying the signal peptide and the mature protein are separated by an intron²⁷. It is interesting that in the *C. t. thummi* globin IV gene neither the signal peptide nor the mature globin is encoded by separate exons.

We have also determined the complete DNA sequence of three other globin genes (designated *A*, *B*, *C* in Fig. 1), including at least 50 nucleotides of their respective 5' and 3' flanking regions. These results are not presented here because sequences have largely been obtained for one DNA strand only. Genes *A* and *B* both code for haemoglobin III, and are transcribed in opposite directions, while genes *C* and *D* encode haemoglobin IV and are also transcribed from different DNA strands (Fig. 1). The deduced amino acid sequences are in complete accordance with those previously reported for haemoglobins III⁷ and IV⁸. As judged from their DNA sequences and putative control regions, genes *A*, *B*, and *C* also represent functional genes and are devoid of introns. In *C. t. thummi* haemoglobin III either threonine or isoleucine is found at position 57 (ref. 7). We find that gene *A* codes for threonine at position 57 and gene *B* for isoleucine. Thus, the two haemoglobin III components are encoded by two non-allelic genes. The derived amino acid sequence of the signal peptide is identical in the four distinct globin genes except for a single replacement (Phe to Leu) at position –13 of globin gene *D*. The tandem arrangement of these four globin genes, which encode two different but closely related globins, reflects the duplication of an ancestral globin gene approximately 40 million years (Myr) ago²¹ and subsequent divergence to globin genes III and IV.

Although the four globin genes investigated have all the characteristics of normal functional globin genes, it might be

Fig. 1 Restriction endonuclease map of the recombinant bacteriophage CttG-1 and sequencing strategy to determine the primary structure of the gene coding for globin IV. *a*, Scale in kilobase pairs (kb). *b*, Restriction map of the 15.5-kb DNA insert. *c*, Enlarged representation of the insert DNA which carries the four globin genes. The boxed segments marked A, B, C, and D represent DNA regions which hybridize to cDNA prepared from larval 8–14S poly(A)⁺ RNA as well as to cloned globin III cDNA. Horizontal arrows indicate the 5' to 3' direction of transcription. *d*, Fine structure and sequencing strategy for the 0.945-kb *Hind*III fragment (D) which carries a *C. t. thummi* globin IV gene. The coding sequence (solid box) and the 5' and 3' noncoding regions (open boxes) are indicated. *e*, Scale in bp for *d*.

Methods: Total RNA was isolated from red *C. t. thummi* larvae as previously described⁵⁰ so, enriched for poly(A)⁺ RNA and fractionated by sucrose gradient centrifugation. Aliquots of the gradient fractions were assayed for the presence of globin mRNA by translation in a cell-free wheat germ system (BRL). ³⁵S-labelled *C. t. thummi* globins were identified by their similar migration with authentic *C. t. thummi* globins on polyacrylamide gels⁵¹. The slightly different mobilities observed for authentic globins and globins synthesized *in vitro* from globin mRNA indicate that probably all *C. t. thummi* globins are primarily synthesized as pre-globins (data not shown, see Fig. 2). Fractions containing globin mRNA were isolated from the 8–14S region of the sucrose gradient and used for the preparation of cDNA. dC-elongated double-stranded (ds) cDNA was prepared and cloned in the dG-elongated *Pst*I site of pBR322²² as previously described²³. Two independent cDNA clones were identified, by DNA sequencing⁵², as coding for *C. t. thummi* globin III. ³²P-labelled single-stranded cDNA (prepared from 8–14S poly(A)⁺ RNA) and ³²P-labelled nick-translated globin III ds-cDNA served as probes for the identification of globin gene sequences within the selected genomic clone, CttG-1⁵³. The restriction map of CttG-1 was established by single and double digestions as well as with partial digests of end-labelled DNA fragments⁵⁴ using the 8.7-kb *Eco*RI–*Xho*I fragment and the 6.8-kb *Eco*RI–*Xho*I fragment, each labelled at the single *Eco*RI site. The 0.7-kb *Eco*RI–*Hind*III fragment (A), the 3.7 kb–*Hind*III fragment (B, C) and the 0.945-kb *Hind*III fragment (D) were subcloned in pBR322 and a detailed restriction map of the hybridizing DNA regions obtained. For the determination of the transcriptional orientation of the individual globin genes, a 5.3-kb *Eco*RI–*Hind*III fragment carrying the four globin genes (Fig. 1c) was derived from the 8.7-kb *Eco*RI–*Xho*I fragment by partial *Hind*III digestion and subcloned in pBR322. The presence of single *Ava*II restriction sites in each of the four globin genes at codon 44/45 allowed the direct determination of the transcriptional orientation by DNA sequence analysis. DNA sequencing was performed according to Maxam and Gilbert⁵², using 5' end-labelled (open circles) or 3' end-labelled (closed circles) DNA fragments. Arrows (*d*) indicate the direction and extent of DNA sequencing.

Fig. 2 DNA sequence of the *C. t. thummi* globin gene coding for globin IV (region D in Fig. 1). The derived amino acid sequence, including the amino-terminal signal peptide, is shown on a line above the coding sequence. Conserved sequences in the 5' flanking region (TATA box) and in the 3' noncoding region (AATAAA) are underlined.

Methods: The 5' and 3' ends of the gene (as defined by the capping site and the poly(A) addition site) were determined by S₁ nuclease protection mapping^{55,56} as previously described²⁹. The capping site of the *C. t. thummi* globin IV mRNA was identified using a 344-bp *Hind*III–*Dde*I fragment 5' end-labelled at the *Dde*I restriction site (codon –3/–2) while the 3' end of the gene was determined with a 287-bp *Hinf*I–*Hind*III fragment 3' end-labelled at the *Hinf*I site (codon 103). After hybridization with larval 8–14S poly(A)⁺RNA, S₁ nuclease-protected fragments were electrophoresed together with Maxam–Gilbert chemical cleavage reactions of the corresponding DNA fragments.

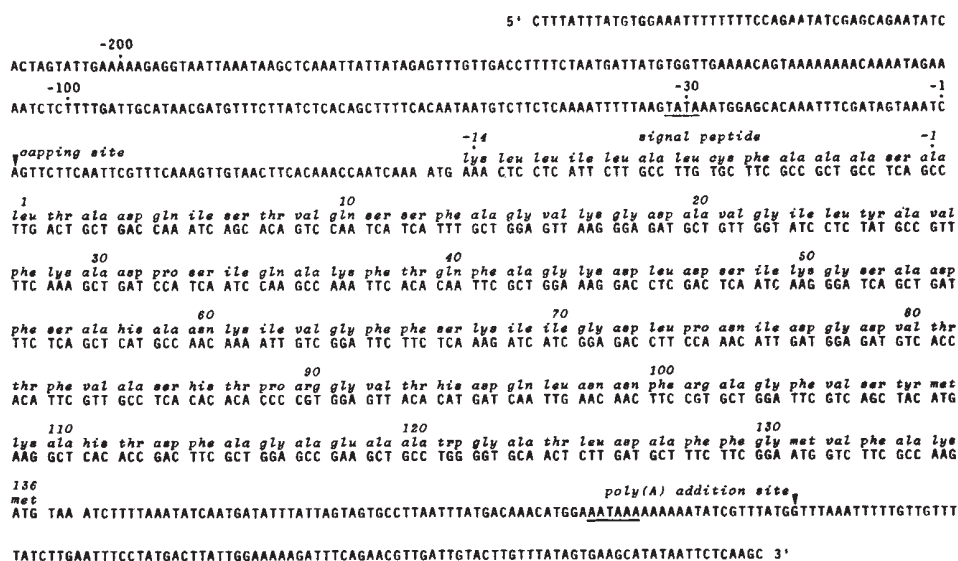
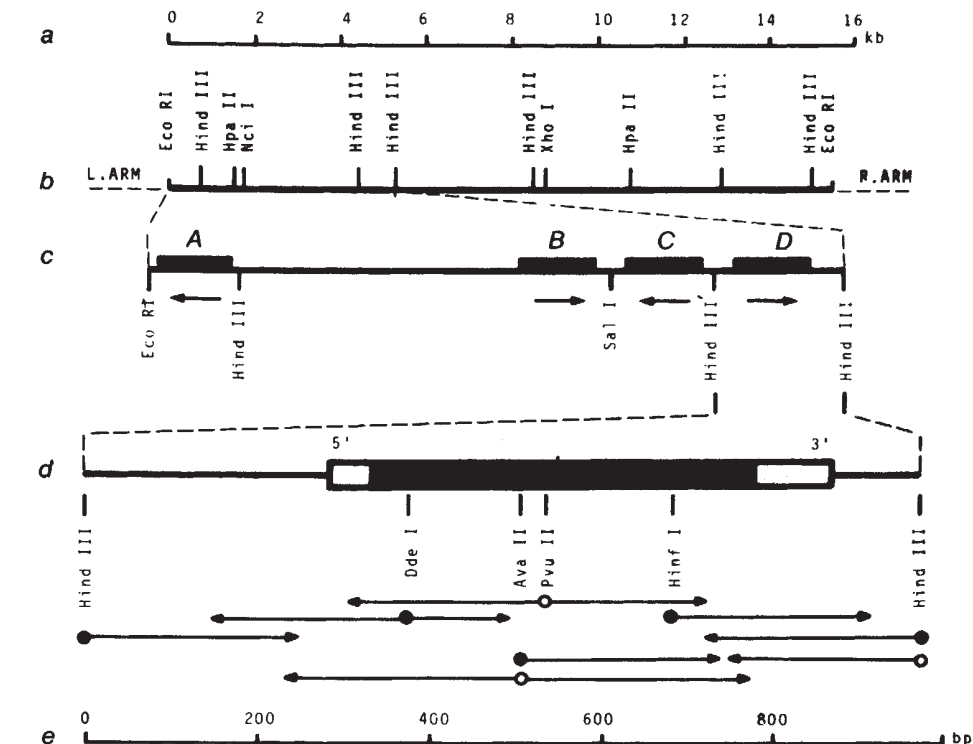
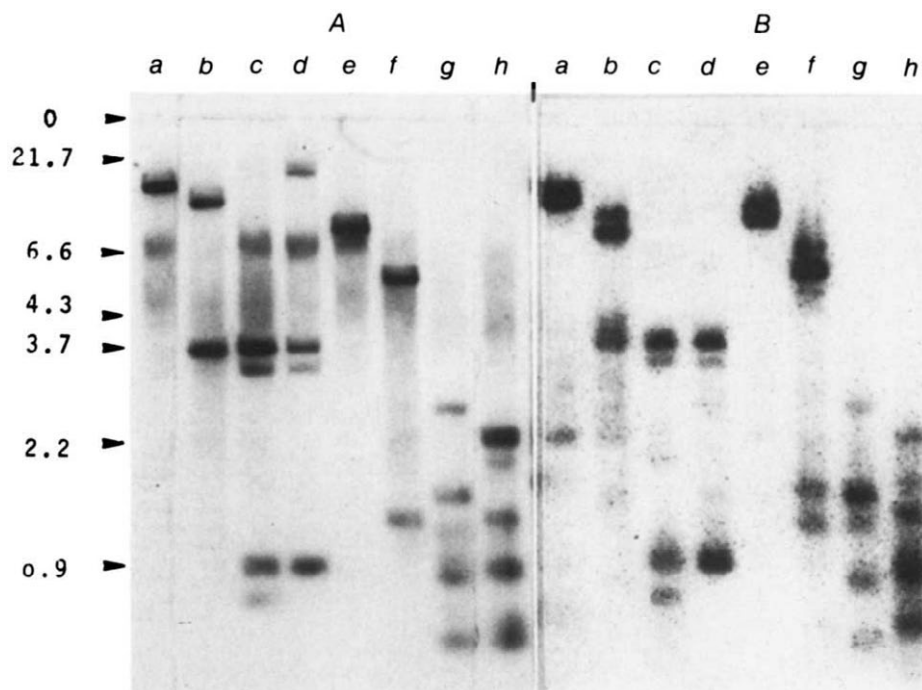


Fig. 3 Restriction and hybridization analysis of *A*, cloned and *B*, uncloned chromosomal *C. t. thummi* DNA. CttG-1 DNA (20 ng) or chromosomal DNA isolated from *C. t. thummi* larvae (10 µg) was digested with various restriction enzymes, electrophoresed on a 0.8% agarose gel, transferred to a nitrocellulose filter⁴³ and hybridized with the nick-translated 0.945-kb *Hind*III fragment (globin gene *D* in Fig. 1). The filter was washed with 2×SSC at 60°C and exposed for 12 h without intensifying screen (*A*) or for 48 h with intensifying screen (*B*). The lanes contained the following DNA digests: *a*, *Eco*RI; *b*, *Eco*RI + *Sall*; *c*, *Eco*RI + *Hind*III; *d*, *Hind*III; *e*, *Eco*RI + *Xho*I; *f*, *Eco*RI + *Pst*I; *g*, *Eco*RI + *Ava*II; *h*, *Eco*RI + *Pvu*II.



argued that they could represent processed pseudogenes, which are not expressed *in vivo*. To investigate this question, we determined the copy number of each of the four globin genes. Restricted uncloned chromosomal *C. t. thummi* DNA, and the cloned DNA insert of the recombinant bacteriophage CttG-1, were digested with various restriction enzymes and analysed by Southern blot hybridization, using the nick-translated 0.945-kb *Hind*III fragment (globin gene *D* in Fig. 1). Figure 3 shows that DNA fragments of the same size hybridize to the globin gene probe in both the cloned and uncloned genomic DNA. These results indicate that each of the four globin genes is probably represented only once per haploid genome. Thus, there appear to be no genes coding for haemoglobins III and IV in the *C. t. thummi* genome other than those carried on the recombinant phage CttG-1. Further evidence for the conclusion that globin gene *D* is transcribed *in vivo* stems from *S*₁ nuclease mapping experiments in which *C. t. thummi* globin mRNA was hybridized to DNA fragments of globin gene *D* extending from the unique *Ava*II site (codon 44/45) to the 5' *Hind*III site and 3' *Hind*III site (see Figs 1, 2). Figure 4 shows *S*₁ nuclease protection for the entire transcription unit of globin gene *D*. *S*₁ nuclease-protected DNA-RNA hybrids terminating within the coding region of globin gene *D* are also observed, probably due to the interference of cross-hybridizing globin III mRNA. We have also compared the DNA sequences determined for the cloned globin III cDNA (codon 76–136, including 22 nucleotides of the 3' noncoding region) with the corresponding DNA sequences of globin genes *A* and *B*. We find identical nucleotide sequences for globin gene *A* and the cloned globin III cDNA while globin gene *B* differs from the globin III cDNA sequence by some silent substitutions in the coding region and a considerable divergence in the 3' noncoding region (data not shown). Thus, by all criteria available, the four globin genes investigated here represent normal globin genes which are expressed *in vivo*.

In Fig. 3, the *Eco*RI-*Hind*III and *Hind*III-restricted CttG-1 DNA (*A*; lanes *c*, *d*) and genomic DNA (*B*; lanes *c*, *d*) show a hybridizing 3.2-kb fragment. This fragment has not been indicated as containing a globin gene in the CttG-1 restriction map (Fig. 1) because it did not hybridize to globin cDNA. These results presumably reflect common DNA sequences in the extragenic region of the hybridization probe (region D) and the adjacent 3.2-kb *Hind*III fragment.

C. t. thummi haemoglobins are relatively slowly evolving proteins which probably arose before the genus *Chironomus*

itself evolved²¹. The lineage leading to vertebrate and insect globins are thought to have diverged ~900–1000 Myr ago¹². One of the ancestral monomeric components²¹, haemoglobin III, has been extensively studied by physicochemical methods^{36,37}. Despite considerable amino acid divergence, the basic structural organization with respect to protein helices, haem pocket and haem contacts is similar in vertebrate and insect haemoglobins.

The unexpected finding of intron-less globin genes is of interest with regard to current ideas on the evolutionary origin of introns as well as to the correlation between exons and protein structural and functional units (see ref. 38 for a recent review). It is not clear at present whether eukaryotic genes acquired their introns during evolution or whether the split gene organization was already present in the common ancestor of prokaryotes and eukaryotes. The three-exon/two-intron configuration of vertebrate globin and myoglobin genes predates the globin-myoglobin divergence 600–800 Myr ago^{1,39,40}. The presence of two introns in the vertebrate globin gene family and of three introns in plant leghaemoglobin genes, however, suggests that a common ancestral globin gene possessed three introns⁶, the third central intron being lost in vertebrates but retained in the plant leghaemoglobin gene. It has been suggested that the leghaemoglobin gene became integrated into the soybean genome after horizontal transfer between insects and plants⁴¹. Our finding that the *C. t. thummi* globin genes lack introns makes this possibility unlikely.

The lack of introns in *C. t. thummi* globin genes cannot easily be explained by convergent evolution of vertebrate and *Chironomus* globins, because comparative amino acid sequence data indicate that the degree of similarity between *C. t. thummi* globin III and human β -globin (16%) is only slightly lower than that found between human myoglobin and human β -globin (23%) or human myoglobin and lamprey globin (19%). Convergent evolution of proteins has been observed for catalytic functions but not so far at the level of amino acid sequence.

An alternative explanation for the lack of introns in the *C. t. thummi* globin genes is that reverse transcription from spliced mRNA in germ-line cells may have produced intron-less cDNA that subsequently became integrated back into the genome^{42–44}. Since neither mRNA nor reverse transcripts contain transcriptional control elements, reintegrated genes are likely to become processed pseudogenes. If, however, the cDNA lines up with the gene from which it originates, a mechanism known as gene conversion (reviewed in refs 45, 46) could remove introns from

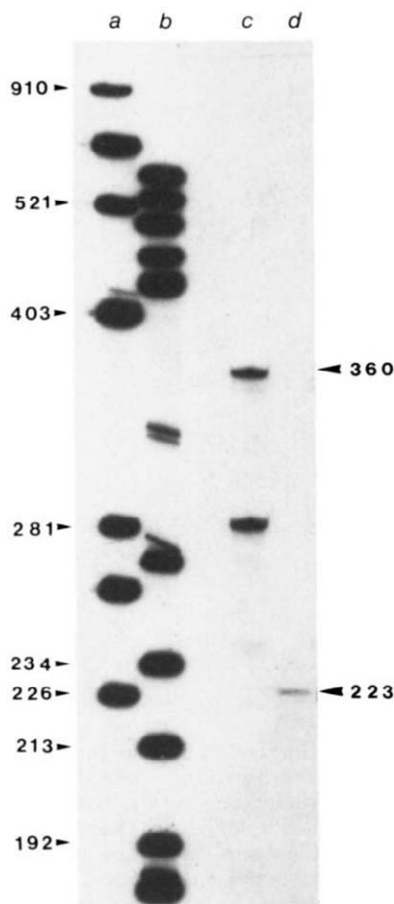


Fig. 4 Globin gene *D* DNA fragments protected from S_1 nuclease digestion after hybridization with *C. t. thummi* globin mRNA. A 482-bp DNA fragment extending from the 5' *Hind*III site to the unique *Ava*II site (codon 44) was 5' end-labelled at the *Ava*II site and a 463-bp DNA fragment extending from the *Ava*II site (codon 45) to the 3' *Hind*III site was 3' end-labelled at the *Ava*II site (see Figs 1, 2). After hybridization with 8–14S poly(A)⁺ RNA, S_1 nuclease-protected^{55,56} DNA–RNA hybrids were analysed on a 6% acrylamide sequencing gel using ³²P-labelled DNA fragments as size markers. Lane *a*, *Alu*I-digested pBR322; lane *b*, *Hae*III-digested pBR322; lane *c*, S_1 -nuclease-protected DNA fragment extending from codon 45 to the poly(A) addition site (360 bp); lane *d*, S_1 -nuclease-protected DNA fragment extending from codon 44 to the capping site (223 bp).

the structural gene without affecting the transcriptional control elements. The elimination of one of the two introns from the rat preproinsulin gene^{47,48} may be the result of an incomplete gene conversion event. The frequency of gene conversion in cells other than those from fungi or yeast⁴⁹, is not yet clear.

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Ring-opened alkylated guanine is not repaired in Z-DNA

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Since the discovery of Z-DNA by X-ray analysis of the alternated hexanucleotide d(C-G)₃ crystals^{1,2}, numerous studies have shown that fragments of natural DNAs can adopt the Z conformation, the topological constraints being a major factor stabilizing this conformation^{3–10}. Immunochemical assays using antibodies to Z-DNA provide strong evidence for the presence of Z fragments in chromosomes^{11–17}. The biological role of Z-DNA is not yet known, but it might be involved in gene regulation. Proteins which bind specifically to Z-DNA have been isolated¹⁸ and interactions between Z-DNA and several cellular proteins^{19–25} have been studied. The ability of DNA repair enzymes to maintain the genome's integrity is of major importance to the cell. On alkylation of DNA by chemical carcinogens such as dimethyl sulphate, methyl methanesulphonate, methyl nitrosourea or methyl nitrosoguanidine, the main target is the N⁷ of the guanosine residue, yielding 7-methylguanine (m⁷G)^{26,27}. In alkaline conditions, the imidazole ring of m⁷G opens up, yielding the ring-opened form 2,6-diamino-4-oxo-5-methylformamidopyrimidine (rom⁷G)²⁸; this lesion is a block to DNA replication²⁹. It occurs *in vivo*³⁰ and is enzymatically removed by a DNA glycosylase³¹. Here we report that the lesion is not excised when present in DNA in the left-handed Z conformation.

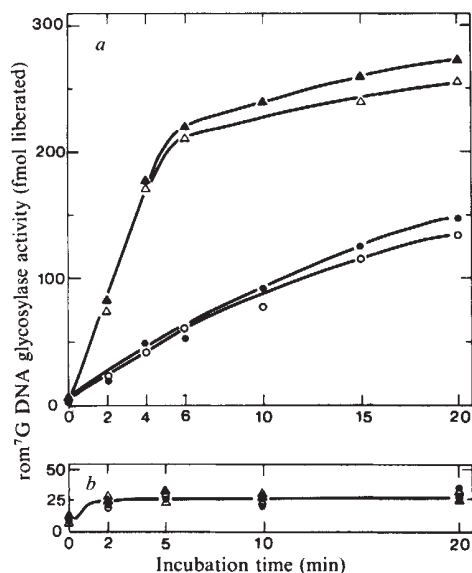


Fig. 1 Activity of *E. coli* formamidopyrimidine-DNA glycosylase using as a substrate poly(dG-dC)·poly(dG-dC) (a) or poly(dG-br⁵dC)·poly(dG-br⁵dC) (b) containing ³H-methyl formamidopyrimidine residues. Open symbols, non-preheated substrates with (Δ) or without (○) added MgCl₂. Solid symbols, preheated substrates with (▲) or without (●) added MgCl₂.

Methods: Poly(dGC·dGC)·poly(dGC·dGC) (PL Biochemicals; 250 μg in 0.3 M Na cacodylate–0.1 M perchloric acid buffer, pH 7.5) was alkylated with 5 mCi of di[³H]methyl sulphate (4.7 Ci mmol⁻¹; NEN) as described previously^{38,39}. With this treatment the polynucleotide contains exclusively m⁷G (97%) and m³G (3%) as modified bases^{26,27}. 3-Methylcytosine and/or O⁶-methylguanine are not detected (less than 0.1%)^{29–32,38,39}. Imidazole ring opening of m⁷G was achieved by alkaline treatment (Na₂HPO₄–NaOH, pH 11.4, 48 h) as described²⁹. In these conditions m⁷G residues are quantitatively transformed into 2,6-diamino-4-oxo-5-*N*-methylformamidopyrimidine (rom⁷G) as checked by HPLC chromatography after acid hydrolysis^{29–32,38,39}. This substrate is abbreviated as rom⁷G-poly[d(GC)] and its specific activity is 6,730 c.p.m. nmol⁻¹. Poly(dG-m⁵dC)·poly(dG-m⁵dC) (PL Biochemicals) and poly(dG-br⁵dC)·poly(dG-br⁵dC), prepared as described previously³³ and containing ³H-methyl rom⁷G, were modified using the same protocol; they are abbreviated as rom⁷G-poly(dG-m⁵dC) and rom⁷G-poly(dG-br⁵dC) respectively. Their specific activities were 7,050 and 6,730 c.p.m. nmol⁻¹, respectively. The percentage of modified guanine residues was ~1%. The rom⁷G-DNA glycosylase was purified from extracts of *E. coli* HB 1100 endo⁴⁰, involving chromatography on Biogel A (0.5 M) and DEAE cellulose, after which the enzyme was further purified by fast protein liquid chromatography using mono-Q and mono-S columns (Pharmacia). The enzyme was purified about 6,000-fold and was free of detectable 3-methyladenine-DNA glycosylase³⁸, uracil-DNA glycosylase³⁹, 7-methylguanine-DNA glycosylase³⁹, apurinic/aprimidinic endonuclease and nonspecific DNase⁴². Details of the purification procedure and properties of the enzyme will be published elsewhere. The activity of rom⁷G-DNA glycosylase in the standard assay was measured by the release of ethanol-soluble rom⁷G from rom⁷G-poly(dGC). Assay mixtures (50 μl) contained 0.38 nmol of ³H-rom⁷G in rom⁷G-poly(dGC), 50 mM NaCl, 5 mM HEPES buffer, 1 mM Na₂EDTA, 10% glycerol (pH 7.6), without or with MgCl₂ at 4 mM, as indicated. After incubation with the enzyme at 37 °C, rom⁷G was separated from the remaining substrate by ethanol precipitation as described for 3-methyladenine³⁸. One unit of enzyme liberates 1 pmol of ethanol-soluble rom⁷G in 10 min at 37 °C in the above conditions. Ethanol-soluble products were identified as rom⁷G by chromatography on Biogel P-2 (ref. 39) or by HPLC³² using authentic samples of rom⁷G as internal standards.

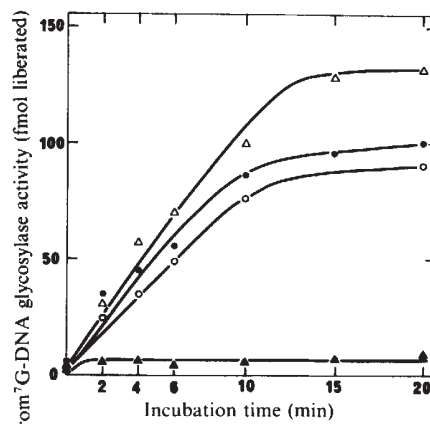


Fig. 2 Activity of *E. coli* formamidopyrimidine-DNA glycosylase using as a substrate poly(dG-m⁵dC)·poly(dG-m⁵dC) in the B or Z form containing ³H-methylformamidopyrimidine residues. The figure shows rom⁷G released by the enzyme at 25 °C from either non-preheated substrate with (Δ) or without (○) MgCl₂ (B form), or substrate preheated without (●) (B form) or with (▲) (Z form) MgCl₂. The substrate in the standard incubation mixture described in Fig. 1 was either unheated or heated (50 °C for 10 min) in the presence or absence of MgCl₂ (4 mM) before enzyme action. Heat treatment is required in the presence of a low concentration of divalent cation to induce the B→Z transition⁴³.

Poly(dG-dC)·poly(dG-dC) was alkylated with di[³H]methyl sulphate and rom⁷G-poly(dG-dC) was quantitatively obtained by alkaline treatment of the alkylated polymer. Figure 1a shows that this modified polymer is a good substrate for *Escherichia coli* rom⁷G-DNA glycosylase. The enzyme activity is increased by addition of 4 mM MgCl₂ to the incubation mixture, whereas heating of the substrate before enzyme action does not modify its activity. In the presence of Mg²⁺ and after heating for 10 min at 50 °C, the modified polymer is in the B conformation as judged by circular dichroism (not shown). It has been verified by chromatographic identification^{29,32} that in all these experimental conditions the enzyme releases more than 98% of rom⁷G (not shown). In the same assay conditions, the enzyme is inactive when the rom⁷G lesion occurs in poly(dG-br⁵dC)·poly(dG-br⁵dC) (Fig. 1b); this is not due to an irreversible binding of the enzyme to Z-DNA, as verified by a competitive experiment using rom⁷G-poly(dG-dC) (data not shown). Note that poly(dG-br⁵dC)·poly(dG-br⁵dC) shows the Z conformation even in the absence of Mg²⁺ (ref. 33). This result suggests that the enzyme is inactive when the lesion occurs in a polymer in the Z conformation.

An experiment using rom⁷G-poly(dG-dC) in the Z conformation is not possible as the enzyme is inhibited by high salt concentrations (≥0.3 M NaCl). To measure the excision of rom⁷G from the same polynucleotide in either the B or Z forms, we used poly(dG-m⁵dC). This polynucleotide has the B conformation in low salt conditions and the addition of small amounts of divalent cation induces the transition from the B form to the Z form¹⁹. In 50 mM NaCl, poly(dG-m⁵dC)·poly(dG-m⁵dC) is in the B conformation. If Mg²⁺ ions (4 mM) are added and the solution is heated at 50 °C for 10 min, poly(dG-m⁵dC) adopts the Z conformation¹⁹. If Mg²⁺ is added without heating to 50 °C, poly(dG-m⁵dC) is stable in the B conformation at 25 °C for over 20 min as judged by circular dichroism (data not shown). This means that the B→Z transition of rom⁷G-poly(dG-m⁵dC) does not occur in the conditions used for the completion of the rom⁷G-DNA glycosylase action.

Figure 2 shows that the enzyme excises the lesion in rom⁷G-poly(dG-m⁵dC) when it is in the B conformation, the activity being increased by addition of Mg²⁺. There is no release of rom⁷G when the modified polymer is in the Z conformation, which confirms the above results obtained for rom⁷G-poly(dG-br⁵dC). Furthermore, the lack of enzyme activity when the lesion occurs in the Z-polymer, is not due solely to heating of the

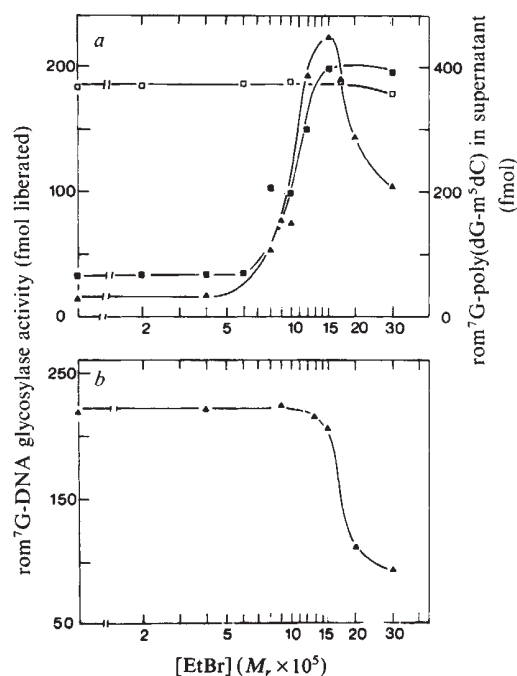


Fig. 3 *a*, Effect of an increase in concentration of ethidium bromide on rom⁷G-poly(dG-m⁵dC) in the Z form as measured by the activity of *E. coli* rom⁷G-DNA glycosylase and the binding of specific rabbit antibodies to Z-DNA. *b*, Effect of an increase in concentration of ethidium bromide on rom⁷G-DNA glycosylase activity when acting on rom⁷G-poly(dG-dC) (in the B conformation).

Methods: rom⁷G-poly(dG-m⁵dC) was converted to the Z form by heating the polymer in the presence of MgCl₂ as described in Fig. 2 legend. Aliquots of the polymer in the Z conformation were supplemented with increasing amounts of ethidium bromide at the indicated concentrations and heated at 50 °C for 10 min to reach conformational equilibrium⁴⁴. The determination of the activity was as described in Fig. 1 legend. Part of each aliquot (50 µl) was treated with excess rom⁷G-DNA glycosylase (50 units, ▲); the remainder was used to measure the binding of either specific rabbit antibodies to Z-DNA (■) or preimmune rabbit antiserum (□). Antiserum to Z-DNA was raised in rabbits immunized with poly(dG-m⁵dC) in which 12% of the guanosine residues were modified on the N⁷ by chlorodiethylenetriamine platinum(II) chloride. This modified polynucleotide is in the Z conformation in physiological conditions³³. The antiserum is specific for Z-form DNA, showing no cross-reaction with the other forms of DNA, and has been verified to have no detectable rom⁷G-DNA glycosylase activity (data not shown). For radioimmunoassay, 12 µl of either the immune or preimmune serum were added to each sample (50 µl). The mixture was incubated at 37 °C for 20 min, cooled to 0 °C and centrifuged at 10,000g for 30 min. The radioactivity of the supernatant (expressed as rom⁷G) was determined and is a measure of the amount of the polynucleotide in the B conformation.

substrate as when located in the B polymer it is a good substrate for the enzyme (Fig. 1*a*).

It has been shown that some intercalating drugs such as ethidium bromide (EtBr)^{34,35} or adriamycin³⁶ can induce a cooperative transition from the Z form to the B form. Figure 3*a* shows that such a transition can also be induced by adding EtBr to rom⁷G-poly(dG-m⁵dC) in the Z conformation. This transition was followed by using antibodies to Z-DNA. The shape of the immunoprecipitation curve of the modified polynucleotide-EtBr complexes is sigmoidal (Fig. 3*a*). At high EtBr concentration, the complexes are no longer recognized by antibodies to Z-DNA. In the same experimental conditions, pre-immune serum fails to precipitate the complexes. In a parallel experiment using the same substrate, we measured rom⁷G glycosylase activity. When the polynucleotide is in the Z conformation, rom⁷G is not excised (Fig. 3*a*), but on induction of the

transition by EtBr, it becomes a substrate for the glycosylase. The enzyme is inhibited at higher EtBr concentration. This inhibition is due to the drug as a similar pattern is observed when a modified poly(dG-dC)-EtBr complex (in the B conformation) is used as the substrate (Fig. 3*b*).

In considering the biological implication of this lack of repair of lesions present in Z-DNA, two main parameters should be discussed: (1) Does alkylation occur to the same extent in DNA in the B or Z conformation? (2) What are the biological implications of the persistence of the lesion in the Z-DNA for the cell?

It has been shown previously that in the reaction of a carcinogen with DNA, the conformation of DNA is an important parameter. For example, the chemical carcinogen *N*-hydroxyaminofluorene reacts with B-DNA but not with Z-DNA³⁵. In our *in vitro* experiments (see Fig. 1 legend), the degree of alkylation by a low concentration of dimethyl sulphate which is biologically relevant is similar for poly(dG-dC) (B form) and poly(dG-br⁵dC) (Z form), as is the opening of the imidazole ring of m⁷G. Dimethyl sulphate reacts equally well with the N⁷ of guanosine residues in B and Z-DNA, as expected from the accessibility and the electrostatic potential of this atom in DNA³⁷.

As it has been shown that rom⁷G occurs *in vivo* and that this lesion is a block to DNA replication, its persistence in a region of DNA in the Z conformation could be a lethal event for the cell. From experiments now underway, we expect to obtain further information on the repair of O⁶-methylguanine (a mis-coding lesion) in DNA of either the B or Z configuration.

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Two types of learning

Sara J. Shettleworth

Adaptive Behavior and Learning. By J.E.R. Staddon.

Cambridge University Press: 1984. Pp.555. Hbk £30, \$59.50; pbk £10.95, \$18.95.

Conditioning and Associative Learning. By N.J. Mackintosh.

Oxford University Press: 1983. Pp.350. £17.50, \$29.95.

AT ONE time, knowledge about simple learning in animals was expected to form the basis for understanding more complex behaviour in all species. That optimistic era has passed, and as a result those psychologists still trying to understand how animals learn are sometimes viewed by their colleagues working on more glamorous problems as inhabitants of a kind of theoretical backwater. But waves have been stirring this backwater lately, waves that are important not only for learning theorists but also for neuroscientists, behavioural ecologists, cognitive scientists and anyone else with an interest in aspects of behaviour involving learning.

Mackintosh and Staddon are both well-known and respected researchers on learning, and their books will be widely read and referred to by other researchers and students in the field. The similarity of the two books ends here, however. Their content, style and aims could hardly be more different. Staddon's book is an attempt, often successful, at a broad and far-reaching account of adaptive behaviour in individuals, ranging from taxes in plants to information processing in human beings. Mackintosh's is a compact and tightly reasoned theory of one historically important and well-studied kind of learning, the formation of simple associations.

In part, the difference in content between the two books reflects a difference between two approaches to research on learning. "Skinnerian" or "operant" research has been concerned with describing the overall, "molar", properties of behaviour when animals are rewarded for performing a simple response such as pecking a disk or pressing a lever. This research has focused on behaviour in the steady state, after the subjects have experienced the same relationship between response and reward for many days or weeks, rather than on the learning process that produces it.

The second approach is to use animals to study the acquisition of associations. Pavlov's dog learning that a bell signalled meat powder was acquiring such an association. Part of this tradition is an attempt to account for the trial to trial, or "molecular", details of the individual animal's behaviour in learning. This approach was given a tremendous stimulus in the 1960s by the development of preparations for studying Pavlovian conditioning that were easy to use with

large numbers of subjects. The past 15 years or so have been a period of active research and theory development in Pavlovian conditioning.

Recently these almost independent sub-areas have been stirred by two common concerns. One is functional and naturalistic. Stimulated by demonstrations that animals can learn either very well or very poorly, depending on how close the required learning is to learning that might be useful to them in the natural environment, psychologists have begun to think about the possible adaptive function and evolutionary basis of behaviour in standard laboratory-learning paradigms. In some cases these thoughts amount to little more than *post hoc* rationalizations for data, the kind of "just so" stories that are the bane of sociobiology. In other cases, however, formal optimality arguments from economics and foraging theory have been applied productively to behaviour in learning experiments.

Considerations of optimality or adaptation are most directly applicable to the products of learning, the behaviour that results from experience. It is somewhat paradoxical, then, that the interest in learning as adaptation has been growing at the same time as the conviction among many studying animals that their subjects are best viewed not as machines that produce responses but as cognitive beings. On this

view, the products of even simple conditioning experiences result from changes in the animal's representation of the world rather than from changes in its stimulus-response connections. Pavlov's dog salivated not because the sound of the bell had become connected to his salivary reflex but because he *knew* that food would follow the sound of the bell. Released from his harness, he would have run to look in the food dish.

In terms of these various currents, Mackintosh's book encapsulates a cognitive approach to associative learning that guides much contemporary work. Only about half as long as his more general *The Psychology of Animal Learning* (Academic Press, 1974), it covers much of the same ground as Anthony Dickinson's *Contemporary Animal Learning Theory* (Cambridge University Press, 1980). But where Dickinson provides a sketch, Mackintosh fills in the details.

In Mackintosh's view, animals form associations between pairs of events in the world (Pavlovian conditioning) or between their own behaviour and its consequences (operant conditioning) according to a single set of rules. The principles of association formation are also the same whether rewarding or punishing events are involved. Likewise, Mackintosh argues, the results of learning that valued events will occur or that they will be absent (excitatory and inhibitory conditioning, respectively) can be embraced in a symmetrical way within a single framework. This scheme appears so simple and satisfactory that one might wonder why it needs even a smallish book to justify it. Historically, however, theories of positively rewarded learning, of punishment and of avoidance learning have all developed somewhat independently of one another. It is therefore necessary to go to



Dog-days of association research — Ivan Pavlov and colleagues at the Military Medical Academy, St Petersburg, around the turn of the century.

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some lengths to show that learning about the consequences of responding occurs in the same way whether those consequences be procurement of food or postponement of noxious electric shock.

The sort of integration achieved by Mackintosh is possible only with a cognitive view of conditioning. If the important result of learning is a change in how the animal represents the world, questions about how learning occurs can be separated from questions about how performance results from it. And even if the laws of association are the same across different types of events, the laws of performance certainly are not. Pavlovian conditioned responses are the result of a process by which the learned signal automatically elicits behaviour related in some way to the event signalled. Signals for food elicit food-related behaviour, for example. But, Mackintosh concludes, no such simple mechanism can account for operant performance. There is no acceptable alternative to viewing it as the result of a kind of process of inference. When a rat has learned that pressing a lever will give it food, it will behave as if obeying the instruction, "press the lever if you want food". Thus if the rat is not hungry or has learned that the food makes it ill, it will (in most cases) no longer press the lever.

This interpretation of instrumental performance sounds a lot like common sense and not much like traditional accounts of why rats press levers. What is astonishing is how robust the non-commonsense theories have been. In much of this book, Mackintosh is reviewing fact and theory in a way which many researchers in the field would find unexceptionable. Nevertheless, it is an exciting book because of the great clarity with which Mackintosh delineates issues which historically have been surrounded by the profoundest muddle and confusion, and argues for his resolutions of them.

Staddon takes a different approach to the tortured history of learning theory — an approach both exasperating and refreshing — and that is simply to present his own synthesis. Except for the most essential, detailed experimental references and issues of mainly historical interest are relegated to extensive notes at the end of each chapter, so allowing Staddon to include an advanced undergraduate text and a specialist book within the same covers.

Many recent books on learning make a bow to the adaptive functions of learning with a chapter or two on bird song, imprinting, foraging as operant behaviour and the like. Staddon is the only author to have attempted a thoroughgoing integration of learning psychology with the biological approach to behaviour on such a large scale. Its starting point is acceptance of explanations in terms of adaptive function on an equal footing with explanations in terms of mechanism. Flying in the face of the tradition exemplified by Mackintosh, Staddon argues that sometimes the best available explanation for a behavioural phenomenon is



Stentor roeselii drawing a cloud of carmine particles into the ciliary disk as part of its normal feeding pattern. The illustration originally appeared in H.S. Jennings's *Behavior of the Lower Organisms* (1906), together with a detailed yet splendidly written account of the reaction of *Stentor* to the irritant. (Reproduced from *Adaptive Behavior and Learning*.)

a functional one. Even when both causal and functional accounts are available, thinking in terms of function may be the more powerful means of integrating data from a wide range of species and situations.

In some areas such as foraging, the function of behaviour can be discussed in terms of a formally defined optimum. However, in places Staddon slips into less rigorous use of functional arguments. For example, grooming in a number of mammals is influenced by food reward much less than are other activities, such as bar pressing. The causal or mechanistic approach to understanding such differences in reinforcement would be to ask whether they are due to differences in the associability of different activities with reward. Or perhaps the animal does not "know" when it grooms the way it knows when it bar presses. Perhaps giving food to hungry animals suppresses their grooming. Attempts to answer such questions experimentally (reviewed by Mackintosh) are fraught with difficulties and may well lead to different answers for different species, different responses or both. Staddon's functional account of these differences is simple: "Evolutionary function is a good guide to reinforceability" (p.443). In other words, since grooming does not usually function to get food in natural environments, we shouldn't expect to be able to reinforce it in the laboratory. In Staddon's terms, the animal's Bayesian prior estimate that grooming produces food should be very low. Given the notorious difficulty of establishing adaptive function, this may strike some as a non-answer. But as other examples in the book show, this kind of approach is exactly the way to make sense of a lot of otherwise puzzling phenomena.

Anyone using this book for teaching purposes will find it includes most of the conventional material in a course on learning, with an emphasis on operant research. But many of the ideas are not standard textbook fare. For example, behaviour in novel situations is thought of

in terms of generation of behavioural variation and selection from it. Bacteria in a chemical gradient are vividly used to introduce this principle. Species differ in the extent to which they rely on merely local memory (Staddon's term for a simple change in responsiveness with time since an event) or more long term, associative, memory. An early chapter introduces feedback and the language of control systems. Within this framework, reinforcement schedules, optimal choice and mechanisms of adjustment to reinforcement schedules are discussed. Formal arguments are used here and, as elsewhere in the volume, students would need a strong quantitative background to benefit from the material.

The chapter on reinforcement schedules will be familiar to anyone who has followed Staddon's work, but other ideas are new and stimulating. For example Staddon argues that the key to a useful comparative psychology is the differing numbers of states of the world species can distinguish. A cat escaping from a puzzle box differs from *Stentor* escaping from a cloud of carmine particles because the cat can distinguish the puzzle box from other situations. It is adaptive for the cat (but not for *Stentor*) to remember its experience in a given situation because it can recognize that situation next time.

As another example, a chapter on memory includes the otherwise unpublished model of spatial memory developed by Dale and Staddon. In what by this point in the book will be a familiar style, Staddon shows that by looking at spatial memory tasks in the right way we can cut across a number of situations that are usually treated separately (here, maze and operant tests of memory) and derive the properties of behaviour in each one from a single set of principles. In this case the required leap is to see the places in a maze as being coded both spatially and temporally and to see the various standard memory tasks as requiring different kinds of discrimination among different kinds of memory traces.

In a book that attempts a broad original synthesis of an established body of research, there are bound to be details to quibble with. However, if the next generation of students were brought up on Staddon's book (along with liberal doses of Mackintosh to sharpen their appreciation for hard-nosed analysis of mechanism) the field of animal learning would surely come to have a very different look. It would merge in a natural way into the study of animal behaviour more generally and new questions about learning would be asked and answered. Whether this is a desirable state of affairs is of course a matter of opinion, but anyone who has ever wondered what it would be like should read Staddon's book. □

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The good old days

R.S. Pease

Cambridge Physics in the Thirties.

Edited by John Hendry.

Adam Hilger: 1984. Pp.209. £17.50, \$30.

NINETEEN physicists contribute their accounts of research at the Cavendish Laboratory during the 1930s. All of them were participants in that great era of discovery which laid the experimental foundations of nuclear physics. Some of the material — the articles by Feather, Chadwick, Cockcroft, Blackett and Oliphant — are reprints which, though covering familiar ground, can well bear repeat performances. Fresh material has been written by Dee, Walton, de Bruyne, Duncanson, Massey, Mott, Dirac, W.B. Lewis, A. Wilson, Allibone, Goldhaber and Peierls.

An introductory framework and a bibliography are provided by John Hendry, whose growing work as a historian

of physics is marked by the extensive and careful use of original source material. As a consequence, the book, though commendably short, presents a substantial canvas, ranging from the background of Cambridge society and personalia, to the substance of the discoveries themselves.

The new accounts of the theoretical physics from Peierls, Massey, Mott and Wilson go far towards redressing the often-accepted view that Rutherford's experimental approach always dominated, and that his team neglected theoretical guidance. Allibone discusses the fruitful involvement of the Metropolitan-Vickers Company in the engineering work and the personnel at the Cavendish. It is a mark of the time that then so little was said of the contribution of the Cavendish to the profitability of industry; but Allibone stresses the widespread subsequent application of the high-voltage and high-vacuum techniques generated by the research. Similarly Wynn-Williams and Lewis stress the application of the electronics developed primarily for

counting. The teaching is illustrated well by de Bruyne, especially in his thumbnail sketch of G.F.C. Searle.

Much of the credit for the success of the Cavendish is attributed to the leadership of Rutherford, whose genius and personality is a feature of almost all the accounts. By contrast Lord Bowden emphasizes the part played by luck. Hendry himself, though providing a background, does not endeavour to penetrate beyond these simple explanations. But the articles themselves, and indeed almost all of the recollections of pre-War Cambridge, illustrate the relative stability and security of that society. Surely this is a contributory factor to the dedication needed for the highest intellectual achievements brought out — albeit not explicitly — especially by those who came from overseas to bring so much to physics at Cambridge in the 1930s.

The strength of this book is the skilful welding of original but rather anecdotal material into a coherent (and very readable) presentation of great research. But the book concentrates on nuclear physics in the early 1930s; consequently there is only passing mention of (for example) the founding of the low temperature physics school, and of the crystallographic work, the development of which into the next great era of Cavendish research was initiated by W.L. Bragg in 1938. □

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Resonance applied

E. R. Andrew

Nuclear Magnetic Resonance Imaging: Basic Principles.

By Stuart W. Young.

Raven: 1984. Pp.163. \$27.

Magnetic Resonance in Medicine and Biology.

By M.A. Foster.

Pergamon: 1984. Pp.244. Hbk £27, \$50; pbk £14, \$25.

NUCLEAR magnetic resonance (NMR), a branch of spectroscopy in the radio region of the electromagnetic spectrum, is well established as an analytical and structural technique in chemistry and physics. In the past decade it has become important in medicine also, in providing *in vivo* anatomical images similar to computed tomography (CT) X-ray scans.

NMR has the merit of producing images of similar resolution to CT X-ray scans, but with superior tissue and pathological contrast, of giving scans not only of transverse sections, but also of coronal and sagittal sections, and of doing all this without hazard since no ionizing radiation is used. Moreover the additional degrees of freedom afforded by tissue relaxation times provide extra dimensions of diagnostic information. As a consequence of these advantages, NMR scanners are rapidly being deployed in hospitals. Diagnostic radiologists and clinicians generally are having to learn about this new technique, which is rather more difficult to comprehend than earlier modalities.

In his book, Stuart Young attempts to explain from scratch the principles of NMR imaging to medical doctors and biologists,

without using mathematics or any depth of physics. It is a daunting task in which the author succeeds rather well with the aid of homely analogies from music and sport; indeed, the non-American reader may learn more about the intricacies of baseball from the analogies with NMR than vice versa. There are many images illustrating the clinical power of NMR, a chapter on site planning and also a rather incomplete listing of systems available. The physicist may complain about some incorrect definitions and an error of 2π in the only equation, but on the whole the book succeeds in providing a straightforward and comprehensible entry into the subject for the physician.

NMR imaging is just one of the important aspects of magnetic resonance. M.A. Foster's book is a more substantial work, covering a wide range of magnetic resonance applications in medicine and biology. The first third is concerned with electron spin resonance and includes a specialist chapter on spin label studies of cells. The remainder of the book is devoted to NMR spectroscopy *in vitro* and *in vivo*, and to NMR imaging, the latter covered in 34 pages in chapters written by Hutchison and Smith. One important area which the author does not attempt to cover is applications of NMR spectroscopy in molecular biology, to the structure and conformation of proteins, nucleic acids and other molecules of biological importance. For the rest, the book is clearly written at a fairly elementary level and should be easily understood by scientists from a wide range of disciplines encountering magnetic resonance in biomedicine for the first time. □

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THEORETICAL PREDICTION OF THE THERMODYNAMIC BEHAVIOR OF AQUEOUS ELECTROLYTES AT HIGH PRESSURES AND TEMPERATURES — PARTS I THROUGH IV

*Harold C. Helgeson,
David H. Kirkham,
and George C. Flowers*

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Man of many worlds

Paul Townsend

Quantum Theory of Gravity: Essays in Honor of the 60th Birthday of Bryce S. DeWitt.

Edited by Steven M. Christensen.

Adam Hilger: 1984. Pp.483. £30, \$54.

ONE of the compliments paid to Bryce DeWitt in this collection of essays is that the strength of his work lies in its "unavoidability"; by this it is meant that "the development of science cannot escape it and, therefore, sooner or later it receives recognition". Overall the book is a good illustration of the remark.

The importance of much of DeWitt's pioneering work in quantum gravity, which is at the root of a number of areas of current research, is only now becoming apparent to many of us who entered the field at a later date. The first four essays in the book chart the course of some of these early ideas in frankly celebratory style, paying tribute to DeWitt's role in their formation or development. These contributions are the kind of idiosyncratic personal history that is naturally absent in journal articles and textbooks and which can be very instructive, even for the reader who is not familiar with the cast of characters. In particular I found the essay by Isham especially interesting as a concise bird's-eye view of the field.

The contributions that follow in the first third of the book take up the theme of quantum field theory in curved spacetime. Among them are accounts of some applications to cosmology and black hole physics, as well as a few that address conceptual problems of this approach. An example of the latter is Davies's provocatively titled essay "Particles do not exist".

The articles constituting the remaining two-thirds of the book grapple, for the most part, more directly with quantum gravity proper. There is no discernible thematic unity here because there is no consensus on the correct way to proceed. As a result, the essays range over a variety of approaches including Hamiltonian methods and the Wheeler-DeWitt equation, renormalization group methods, supergravity, and two- and three-dimensional spacetime analogues of quantum gravity, with several excursions into higher derivative modifications of general relativity.

There is only one contribution devoted to what is perhaps DeWitt's most fruitful creation, the "background field method", but it is a remarkably good one; this is the essay by Vilkovisky with the rather uninformative title "The Gospel according to

DeWitt". A later article by Stelle reminds us of the power of this method in applications to supersymmetric field theories, supergravity in particular, although the latter subject is represented only briefly. Not represented at all is the "Kaluza-Klein" approach, currently very popular in its conjunction with supergravity, but neither of these topics has been influenced greatly by Bryce DeWitt. Finally, towards the end of the book, there is an essay by Smolin reminding us that DeWitt is one of the few physicists who have taken more than a passing interest in those quasi-philosophical problems that bedevil the interpretation of quantum mechanics; he is a well-known advocate of the bizarre "many worlds" interpretation.

On the whole I have the impression that the contributors have made a serious effort to write stimulating, readable essays, and this immediately puts the book in a category above most conference reports on quantum gravity. Also, discounting conference reports, I believe that this is the only collection of essays on quantum gravity extant, probably because no other figure of comparable stature has yet reached the age of 60. While similar volumes will surely follow, this one is likely to remain a valuable source for those interested in the early years of quantum gravity. □

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Unity in diversity?

Athel Cornish-Bowden

Biochemical Adaptation.

By Peter W. Hochachka and George N. Somero.

Princeton University Press: 1984. Pp.537.

Hbk \$60, £60; pbk \$19.50, £15.

ALTHOUGH biologists have always been impressed by the diversity of life, biochemists have, on the contrary, emphasized its essential unity. All organisms use the same metabolites, arranged into the same series of metabolic reactions, catalysed by similar enzymes synthesized by the same kind of genetic apparatus. Biochemists have found that they can concentrate on a few organisms, and have done so to the extent that biologists have been known to complain that *Escherichia coli* is the only animal they know.

Nonetheless, life exists not only at 37°C at atmospheric pressure in the presence of water and oxygen, but also in many other habitats that might appear impossibly hostile, such as hot springs, frozen deserts and the ocean depths. Even quite familiar animals can endure conditions that one might suppose to be lethal: the goldfish, for example, can survive in the complete absence of molecular oxygen for several days.

The pioneer in the field of biochemical diversity was Ernest Baldwin, whose little book *An Introduction to Comparative Biochemistry* (published by Cambridge University Press in 1970, but dating originally from 1937) still provides a stimulating entry into the subject. Much has been learned since his time, and Peter Hochachka and George Somero have written a much larger book, *Biochemical Adaptation*, with the aim of making biochemists more aware of the vast range of habitats in which life is possible and of showing how the diversity can nonetheless be rationalized in terms of a single biochemical theme.

I believe the authors to have been successful in presenting the range of their subject. But I am far from happy with their rationalization, which seems to stretch theoretical chemistry to breaking point in many instances. The chapter on temperature adaptation gives prominence to entropy-enthalpy compensation, the idea that the enthalpies of activation of enzymes catalysing the same reaction in different organisms vary linearly with the corresponding entropies of activation. Some authors have apparently even found a mystic significance in the fact that the slopes of "compensation plots" are often close to 310 K (i.e. 37°C). Yet nowhere is there any recognition of the fact that the numbers plotted can only be obtained by extrapolating to absolute zero a trend measured over perhaps 5°C to 40°C, an extrapolation some eight times the range of measurement! Artificial correlation between slope and intercept is thus inevitable regardless of whether there is a real physical phenomenon.

Elsewhere in the same chapter, the authors speculate on possible reasons why such enzymes as lactate dehydrogenase may be more "efficient" in some organisms than in others. This may be a meaningless question how ever it is formulated; it is certainly meaningless when the "efficiencies" span a range as small as 0.54 to 1.00, when they are defined in terms of the rate at substrate saturation and not at a physiological substrate concentration, and when they refer to a standard temperature that is unphysiological for some of the enzymes compared.

In the words of Theodosius Dobzhansky, "nothing makes sense in biology except in the light of evolution". Evolution makes only fleeting appearances in the book, but it might have been more convincing as a central theme than an appeal to physical chemistry which seems premature given our present state of knowledge. □

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To coincide with the Third European Congress on Biotechnology, we feature here twenty five new products of interest to the biotechnologist, including a new range of purified antibodies

● The ChemLab Micromedic reagent dispenser is designed to dispense small volumes of biological materials. The dispenser, is capable of delivering selected volumes over a range 1 μ l to 9,999 μ l in one microlitre increments with a repeatability of $\pm 0.5 \mu$ l and accuracy of 1%. The liquid to be dispensed is held in a cartridge in the reagent dispenser so that only a minimal amount of the reagent is required to prime the cartridge and there is virtually no wastage.

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● The Genex manual solid phase kit for the production of oligonucleotides includes all the reagents necessary for the synthesis of oligonucleotides, a reaction vessel, ancillary hardware, assembly instructions and detailed protocols for synthesis using phosphotriester chemistry (which have been fully tested). The synthesis kit enables laboratory personnel with limited experience in chemical synthesis to produce an oligonucleotide in a day. The quantity of HPL grade mononucleotides and reagents supplied with the kit is sufficient for 70 couplings, and additional reaction vessels and hardware are available to expand the system for several simultaneous syntheses. Genex recommend using only the Genex HPL grade dinucleotides, since the time that is required for synthesis can be halved without reaction efficiency being impaired.

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● A new fact sheet from Schleicher and Schuell describes the S&S Mini-Collodion Membrane designed for quick, efficient, and simultaneous concentration and dialysis of protein and nucleic acids in small sample quantities. Features of the new membrane include a microtapered collection tip that yields high sample recoveries of up to 98% with concentrations down to 10 μ l. The Mini-Collodion Membrane has a volume of 2 ml and molecular weight cut-off at 25,000. Simultaneous concentration and dialysis is achieved simply by using the membrane in conjunction with an easy-to-assemble S&S all-glass apparatus although the membrane can also be used without the specialized apparatus for dialysis. The new membrane requires minimal physical manipulation ensuring virtually no sample loss, and rigid design allows the membrane to be washed and reused. Shorter sample preparation times are achieved because of the greater surface area-to-volume ratio than that of the standard 8 ml S&S collodion membrane.

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● A complete selection of solid supports and nucleosides for oligonucleotide synthesis is available from J. T. Baker Research Products. To ensure easy handling and high yields of the desired oligonucleotide chain, Baker's supports are silica-based, with a large particle size (40 μ m) and variety of pore sizes (60Å, 250Å, 650Å) available. Baker performs its own bonding and derivatization to provide stable forms of succinoyl-aminopropyl (SAP) silica gels and DMT, N-protected nucleoside-derivatized-SAP-silica gels. In addition, the actual analytical values for nucleoside loading (in μ mol per g) or amine loading (in meq per g) are provided on the label of every bottle to facilitate economical and successful use. A variety of high purity nucleosides is also supplied by Baker Research Products; included are nucleosides, N-protected nucleosides and 5'-DMT-protected nucleosides. These products are suitable for use in either phosphotriester or phosphoramidite synthetic methods after further derivatization, and are completely compatible with the Baker solid supports.

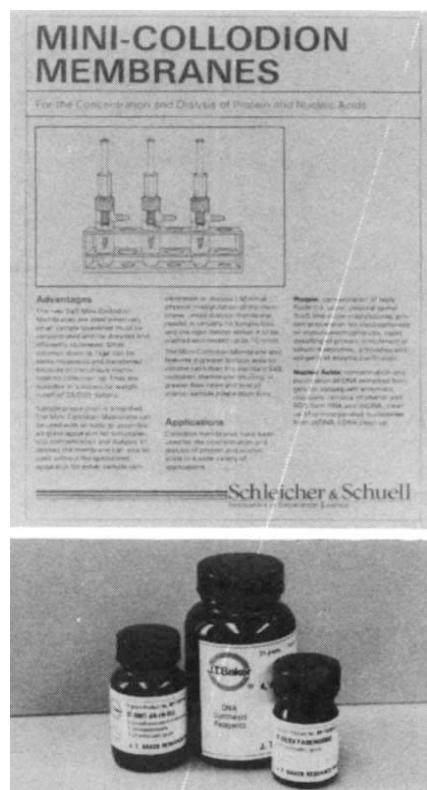
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● Neuropeptide Y (NPY), is currently available in synthetic pure form from Bachem UK. The pure lyophilized form is available from Bachem UK (as are PYY, PHI, VIP, pancreatic polypeptide and a large range of other important peptides), with full technical support including a free quality-control service (using HPLC FAB-MS and NMR techniques).

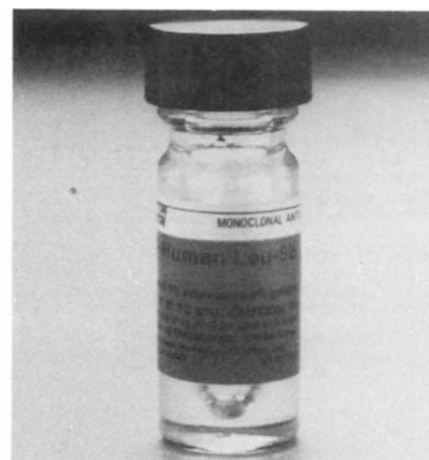
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● Anti-Leu-5b, a new and potent complement-fixing monoclonal antibody which binds to the sheep erythrocyte (E) receptor, is now available from the Becton Dickinson Monoclonal Center. This antibody (Clone S5.2) reacts with 95-100% of E+ cells in peripheral blood, 83% of peripheral blood lymphocytes, 96-99% of thymocytes, and a subset of NK cells, but not with B cells or monocytes. The reagent has been successfully used to enumerate or deplete T cells in peripheral blood, and to identify T cells in tissues. Anti-Leu-5b is available in both purified and FITC conjugated forms for use in direct cytotoxicity, indirect immunoperoxidase, and direct immunofluorescence procedures.

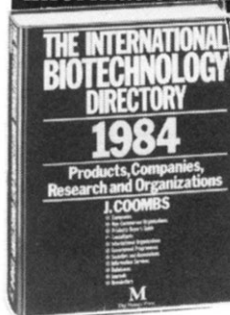
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Fact-sheet on Schleicher and Schuell Mini-Collodion Membrane (top left), Anti-Leu 5b from Becton Dickinson (top right), a selection of nucleosides from J.T. Baker (bottom left) and Genex solid phase kit (bottom right)



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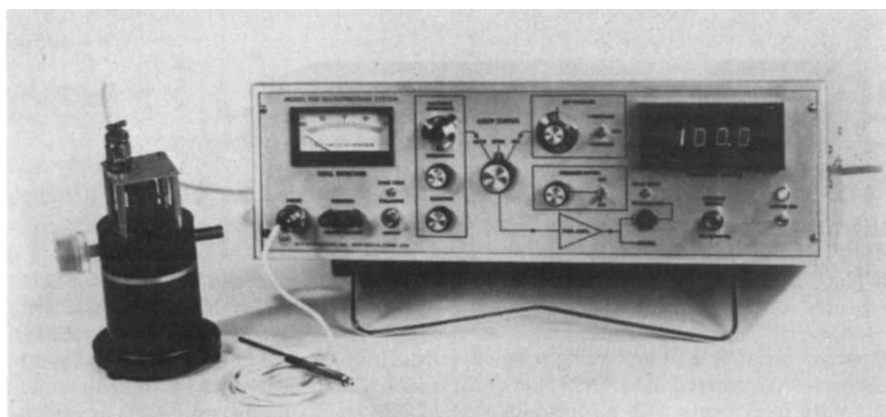
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● An Immunofixation Electrophoresis (IFE) assay kit has been introduced in the Paragon Electrophoresis product line by Beckman Instruments, Inc. simplifying immunofixation electrophoresis in clinical research. The new paragon IFE test provides a fast, sensitive and easy to read method for diagnostic identification of monoclonal gammopathies, immunoglobulin deficiencies and other immunoglobulin anomalies; IFE is a procedure combining protein electrophoresis and immunoprecipitation. Paragon IFE is only one of nine electrophoresis assay kits now available from Beckman. Others are Acid Hemoglobin, Hemoglobin, Serum Protein Electrophoresis (SPE and high resolution SPE-II), Lactic Dehydrogenase Isoenzyme (LD), Creatine Kinase Isoenzyme (CK), Immunoelectrophoresis (IEP) and Lipoprotein. Also, available are electrophoresis controls for SPE (normal and abnormal), LD, CK and Hemoglobin, and complete test processing equipment—power supply, incubator, dryer, wet processor, gel frames, applicators and pipet tips.

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● New from Sera-lab are: catalogue no. MAS 100 mouse IgG, antibody recognizing a determinant on the alpha chain of rat Ia antigen, homologue of mouse Ia-E product (Clone OX 17); MAS 101 mouse IgG, antibody recognizing a monomorphic determinant of rat class I MHC antigens (Clone OX 18) and; MAS 102, mouse IgG antibody against high molecular weight form of rat leukocyte-common antigen (Clone OX 22). These products are available in supernatant and ascites form.

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● Monoclonal anti-human kappa (Clone TB28), a new antibody from Becton Dickinson, is specific for the kappa light chains of all classes of human immunoglobulins and Bence Jones kappa does not react with lambda light chains, giving superior results in tissue staining of frozen sections. Anti-kappa reacts with 50 to 70% of normal B lymphocytes and with Ig² leukaemic cells, and can be used to study the clonality of B cells in tissues by indirect immunoperoxidase techniques.

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● Fibroblast growth factor (FGF) and extracellular matrix (ECM) coated culture dishes facilitate *in vitro* cultivation of cells with fastidious nutritional requirements and provide a means to develop new cell lines. The dishes are now available from Associated Biobabs. ECM is a naturally produced basement membrane-like substance which, unlike reconstituted matrixes, has its various components in their native configuration and proportion. Cells plated on ECM exhibit higher plating and cloning efficiencies, have longer life-spans, reach a higher saturation density and have lower requirements for serum and added growth factors. They also respond better to physiologically occurring hormones and growth factors, express differentiated properties and attach and migrate rapidly. FGF is mitogenic to a variety of mesoderm derived cells at nanogram levels. Use of FGF in cultures accelerates cell proliferation, increases the colony-forming and plating efficiency by allowing cells to proliferate at clonal cell densities and permits expression of differentiated characteristics. FGF comes as a purified, water soluble powder.

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● The model 900 Micropressure system from World Precision Instruments is designed to measure hydrostatic pressure in limited volume biological compartments such as small vessels and cells. The sensing element of the system is a fluid-filling glass microelectrode with a tip diameter range of 2 to 5 microns. The system includes microelectrode holder, preamplifier, power amplifier, hydraulic servosystem, pressure transducer, and digital pressure readout.

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● Bioengineering UK has launched a new rack-mounted instrumentation package for laboratory fermenters. Although the measurement and control modules making up the package are mounted they can easily be unplugged from the rack and used as stand-alone units, a feature made possible because each module has its own power socket and digital readout. The modules cover a wide range of parameters including pH, oxygen, carbon dioxide, massflow, pressure weight and redox as well as basics such as temperature and agitation speed. Ease of use and safety are two features well covered by the new package. For example, the temperature module is equipped with safety cut-outs which prevent overheating, as well as warning lights should a pump or heater failure occur. The temperature controller comes as standard with a semi-automatic sterilization facility with variable, user set values, for temperature and time of sterilization. When this module is used in conjunction with the patented Autosteril system for *in situ* sterilization of the inlet air filter, sterilization is initiated by simply pushing a button.

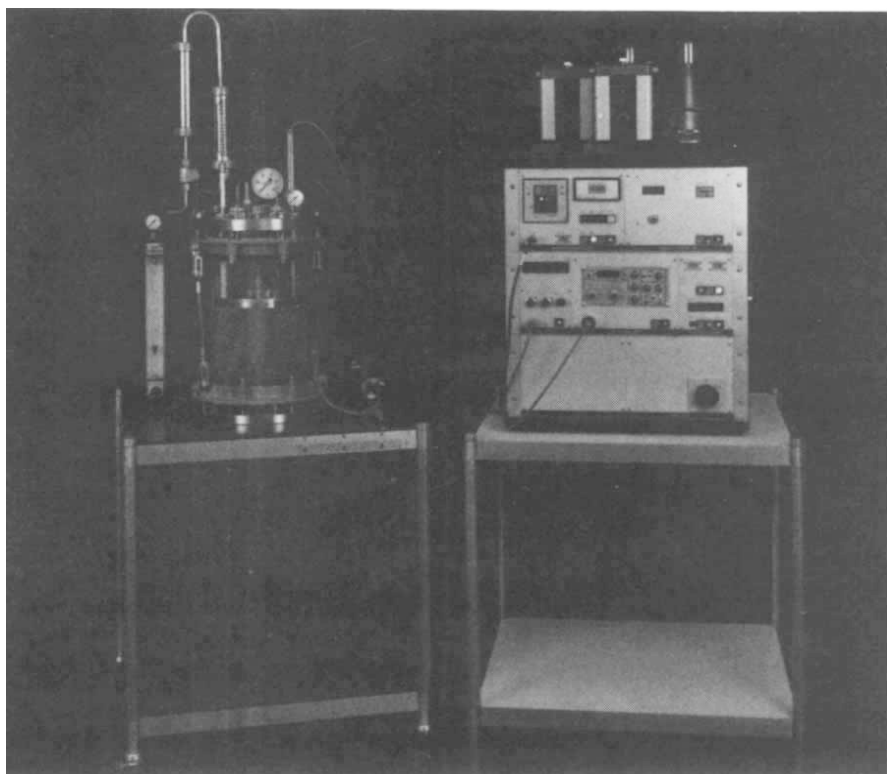
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● BioMag functionalized magnetic particles from Advanced Magnetics Inc. are supplied either as an amine terminated particles suitable for the attachment of biologically active molecules, or with specific binding proteins covalently linked to the particle. Specific binders available include protein A, goat anti-rabbit IgG, goat anti-mouse IgG, and charcoal. When used in conjunction with BioMag's magnetic separator or magnetic test tube rack, these products enable the investigator to make separations rapidly, conveniently, and safely without the columns, centrifuges, and filters now used in conventional solid phase separation systems.

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● Batch-to-batch variation and other problems related to cell growth in the presence of serum can be overcome by the use of a new serum-free supplement, marketed by LKB Instruments, Inc., of Gaithersburg, Maryland. Ultrosor G is a true fetal calf serum (FCS) substitute for *in vitro* multi-purpose cell culture. It contains six main groups of substances, all of them essential for eukaryotic cell growth. These groups are growth and adhesion factors, mineral trace elements, vitamins, hormones, and binding proteins. The optimum concentration of each constituent has been determined according to the needs of a number of cell types. Constancy of composition, both qualitative and quantitative, ensures excellent batch-to-batch reproducibility. All components of the mixture — water and buffer elements included — are selected and assayed under rigorous conditions, thereby ensuring the high quality of the final product, and the growth and adhesion factors are prepared using new extraction techniques including purification by affinity chromatography.

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Rack-mounted instrumentation package for laboratory fermenters from Bioengineering with easily unplugged measurement and control modules

● Continuous culture of clean monoclonal antibodies in a serum-free medium is possible with the Techne CS-100 Cytostat. The Cytostat keeps hybridomas producing monoclonal antibodies, interleukins, growth factors and so on at exponential growth rates by continuously extracting cell products while adding medium at a rate that matches the growth rate of the cells. It is an integrated unit with 4 × 1000ml culture flasks with heavy stainless steel caps and floating stirrers, a constant temperature water bath, an absorption-type refrigerator, 4 peristaltic pumps and sterilizable tubing all connected and ready for use. The system is mounted on a sturdy stainless steel laboratory cart with wheels which can be locked when necessary and control panels between each of the two pairs of peristaltic pumps provide mains power switches for the pumps, water bath, refrigerator and magnetic stirrers with electronic speed control. The absorption-type refrigerator, included with the Cytostat, has a convenient top lid and is built on horizontal slides so that it can be rolled out to gain access to the flasks containing the medium. Also supplied with the Cytostat is a syringe unit to enable simple withdrawal of cell culture medium for sampling purposes.

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● Opti-MEM I, manufactured by Hybridoma Sciences Inc., is a low serum cell culture medium specially suited to the growth of hybridoma cells and the purification and production of monoclonal antibodies. Distribution in the United States is through Gibco Laboratories.

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● New, disposable polystyrene MIC plates and inoculum trays designed to be compatible with virtually all manual and automated 96-channel reagent dispensing systems are now available from Bellco Biotechnology. MIC plate well capacity is 0.3 µl; well diameter is 6.4mm. Each well is clearly numbered and lettered for quick reference, and an extended rim around each well prevents interchange of reagents. An extra thick base rim with non-slip surfaces facilitates handling and stacking. Inoculum tray 200ml capacity and built-in 100ml calibration permit time-saving direct dilution. High 12mm walls and a wider top with an anti-sloshing edge ease handling. Plates and trays are radiation sterilized to eliminate ethylene residue; each lot is tested, for sterility and packaged in quantities of five in plastic tubs sealed to maintain sterility. Cluster plate sealers are also available.

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● A new autoradiography grade of 2,5 diphenyloxazole (PPO) from Biotechnology Supplies has been designed to assure optimum enhancement in gel fluorography. Specially controlled and tested, PPO is now available for agarose and polyacrylamide gel band visualization using the classical Bonner and Lasky enhancement procedures. Optimum resolution, sensitivity and costs are assured for publication quality when autoradiography grade PPO is used according to protocols. Material is available for immediate shipment, conveniently packaged to yield 1, 2 or 4 litres of autoradiography fluid, or in the 5 kilogram economy pack.

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● A high-capacity, simple to use device for electrophoretic transfer of protein or nucleic acids to nitrocellulose or other derivatized papers has been added to American BioNuclear's product range. The EBU-1000 features over 92 inches of platinum wire mounted in adjustable electrode panels. This design ensures even current distribution across the gel for uniform transfer of macromolecules. The transfer chamber is constructed to accommodate up to three gels simultaneously, each with dimensions as large as 15×21.5 cm. An optional cooling tube is available for convenient regulation of temperature. The system is easily disassembled for cleaning and storage.

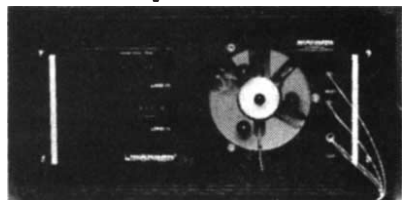
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● SeaPrep agarose, developed for efficient culture and recovery of viable cells or clones, is now available from the Bio-Products Department of Marine Colloids Division, FMC Corporation. This 'ultrasoft' agarose can be used in hybridoma cloning, protoplast cloning, and improved viral plaque assay techniques. Its low gelling and melting temperatures allow SeaPrep agarose to be used safely with heat labile materials, and the high gel clarity simplifies identification and recovery of individual cells and clones; its low melting temperature facilitates transfer to liquid culture media.

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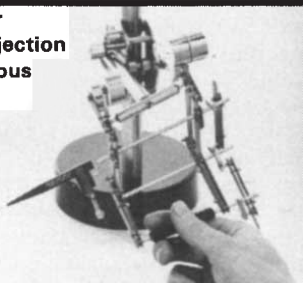
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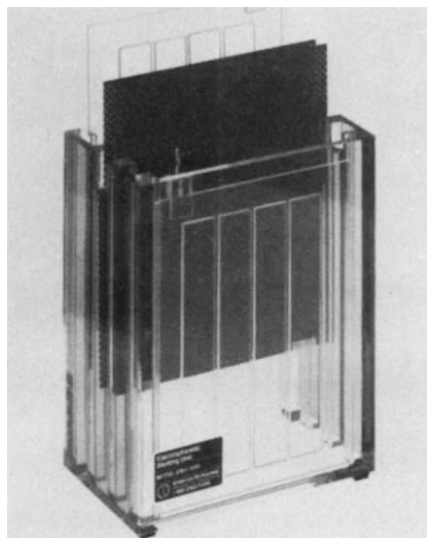


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Electrophoretic aid from American BioNuclear (left) and SeaPrep agarose from FMC (right)



● The new Tago radial immunodiffusion (RID) systems are intended for the quantitation of the individual IgG subclasses of monoclonal antibodies in mouse hybridoma ascites. Also available is an RID system for mouse IgM. These Diffu-Gen assays, based on gel immunoprecipitation, are specific, reproducible, and simple to perform. Each agarose plate has 12 application wells and contains an antiserum specific for the H chain determinants of IgG₁, IgG_{2a}, IgG_{2b}, IgG₃, or IgM. Subclass cross-reactivity has been minimized by affinity chromatography adsorption. The actual range of the RID plates allows the quantitation of monoclonal antibodies between approximately 5 and 100 mg dl⁻¹. Samples of ascites are used at dilutions up to 1:10. Samples of cell culture supernatants may be detected, but not accurately quantitated, if lower than 5 mg dl⁻¹.

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● Triton Biosciences Inc. announced the introduction of affinity purified antibodies to an oncogene protein and a transforming growth factor. Each antibody is generated against a synthetic peptide corresponding to an amino acid sequence in the native protein. These antibodies are purified by affinity chromatography, thus reducing non-specific reactions for clearer, faster results. Western blot and immunoprecipitation analyses are used to verify their reactivity and specificity. These two new antibody products will not only be useful in cancer research, but they may also be used to elucidate the mechanisms in the regulation of normal cell growth and differentiation.

Anti-ras^{Hap21} antibody is the first in a series of oncogene analysis products that Triton is producing. Antibodies to oncogene proteins may be used in semi-quantitative studies that will help clarify changes in oncogene expression. Ultimately, these antibodies could be used in simple diagnostic tests to detect human malignancies.

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● The new Immunoreader NJ-2000 from Tissue Culture Services can be used either in enzyme immunoassays or in other assays where optical determinations are involved. The NJ-2000 is microprocessor controlled and can be programmed to read and analyse various parameters as required. With the addition of an optional autoloader to the basic unit, the NJ-2000 has the capacity to read up to 15 microplates in sequence, and it can be connected to various data processing systems via a computer interface (RS 232C or GP-1B).

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● The American Type Culture Collection now offers two cell culture feeder populations for use in research and hybridoma isolation. MRC-5 is a human diploid lung cell line, and STO a mouse embryonic fibroblast cell line. The cells are gamma irradiated to inhibit cell division and are cryopreserved. Feeder cells are used to support the growth of fastidious cell populations such as primary cells, hybridomas and cells with low colony forming efficiency. They retain their metabolic properties for one to two weeks, and can support the growth of other cells by conditioning the culture medium or through direct cell contact. The MCR-5 and STO feeder cells are available as frozen suspensions. Each ampule contains a sufficient cell population for seeding feeder layers to as much as 225cm² of culture vessel surface. Instructions are provided for thawing, recovering and proper seeding of the feeder cells. Feeder layers prepared from these two cell lines have been in use at ATCC for the past several years to maintain various primary epithelia and to support growth of certain hybridomas and other cell lines.

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These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.